Trends in polychlorinated biphenyl residues in three British predatory bird species.

Thesis

Trends in Polychlorinated Biphenyl Residues in Three British Predatory Bird Species

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Declaration

The work embodied in this thesis was carried out by the author between July 1997 and July 2006 at the Centre for Ecology and Hydrology (CEH), Monks Wood, Cambridgeshire. This thesis was completed under the supervision of Dr R. F. Shore and Dr D. R. Roberts.

The work draws upon data collected and produced as part of CEH's Predatory Bird Monitoring Scheme; consequently the collection of samples, recording of provenance data and the post-mortem examination of all birds has been carried out by dedicated members of staff other than myself. Additionally from 1996 onwards, the sample processing for chemical analysis was carried out with the assistance of a laboratory technician under my supervision.

I declare that except where stated above the work presented is the result of my own investigations. The material within this thesis has not been submitted, nor is currently being submitted, for any other degree.

Parts of the work have been published/presented as listed below.

Wienburg, C. L. and Shore, R.F., 2004. Factors influencing liver PCB concentrations in sparrowhawks (*Accipiter nisus*), kestrels (*Falco tinnunculus*) and herons (*Ardea cinerea*) in Britain. *Environmental Pollution* 132, 41–50.

SETAC Europe 16th Annual Meeting, 2006. The Hague, Netherlands. Oral Presentaion by Dr R. Shore. *Does nutritional state mask long-term contaminant trends in predatory birds?*

3rd International Wildlife Management Congress, Christchurch, New Zealand 2003. Poster presentation. C. L. Wienburg and R. F. Shore *Major factors affecting PCB concentrations in predatory birds in Britain.*

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Dedicated to my parents Denise and Barry, for their help, encouragement and devoting their retirement to their granddaughter in order that I might complete this thesis. I love you both.

To Casper with much love – for his uncharacteristic patience, thank you for waiting.

Abstract

Liver total PCBs have been monitored in British sparrowhawks *Accipiter nisus*, kestrels *Falco tinnunculus* and herons *Ardea cinerea* since the 1960s. Concentrations do not appear to have declined in sparrowhawks or kestrels, despite bans and restrictions on PCB manufacture and disposal, but residues vary markedly between individuals which may obscure long-term trends. In this study measurements were made of PCB liver concentrations in sparrowhawks, kestrels and herons collected from locations throughout Britain between 1992 and 1997: (i) to determine the major causes of intra-species variation in liver concentrations, and (ii) to characterise and quantify liver concentrations of individual congeners and their associated toxicity. The long-term PCB data were also reanalysed to determine if there was evidence of declines in liver PCBs once intra-species variation was accounted for.

Body condition, and to a lesser extent age and sex, affected liver PCB congener, sum PCB and Toxic Equivalent Quotient (TEQ) concentrations. Concentrations were higher in starved than in non-starved birds, largely because of remobilisation of residues (sparrowhawks and herons) and starvation-induced liver wastage (all species). Adults generally had higher liver PCB concentrations than first-year birds, and male sparrowhawks and kestrels lower concentrations than females, although this varied with body condition. Congener profiles were similar across species, and concentrations of individual congeners were primarily determined by their potential to be metabolised. PCBs 138, 153, 180 and 170 accounted for up to 76% ∑PCB concentrations. Non-*ortho*, coplanar PCBs (primarily 118 and 169) were mostly detected in starved birds. Liver TEQ concentrations were below Lowest Observable Effect Concentrations. Intra-year variation in body condition obscured a decline in liver PCB concentrations in sparrowhawks, but liver PCB concentrations have not declined in kestrels.

The results obtained provide insights into which factors should be taken into account when using tissue concentrations in biota to monitor changes in environmental exposure to contaminants.

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Abbreviations

AD	Anderson-Darling
A*h* receptor	aryl hydrocarbon receptor
CEH	Centre for Ecology and Hydrology
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethene
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
EURING	European Union for Bird Ringing
GIS	Geographical Information Systems
GLM	general linear model
GSE	geometric standard error
HEOD	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene (dieldrin)
ICES	International Council for the Exploration of the Sea
IUPAC	The International Union of Pure and Applied Chemistry
LOEC	lower observable effect concentration
LOEL	lowest observable effect levels
lw	lipid weight
ND	non-detected
NOEC	no observable effect concentration
OC	organochlorine
OS	Ordnance Survey
PBMS	Predatory Bird Monitoring Scheme
PCA	principal components analysis
PCB	polychlorinated biphenyl
POP	persistent organic pollutants
SE	standard error
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	toxic equivalent factor
TEQ	total equivalent quotient
UNEP	United Nations Environmental Program (UNEP)
WHO	World Health Organization

Contents

Chapter One

Introduction

1.1 Polychlorinated biphenyls

1.1.1 Structure and nomenclature

Polychlorinated biphenyls (PCBs) are a group of synthetic compounds of the general formula $C_{12}H_{(10-n)}Cl_n$ where n can be up to a maximum of 10. They were first produced commercially during the 1930s by chlorination of biphenyl in the presence of a catalyst such as iron chloride (Robinson and Lenn, 1994). The degree to which chlorination occurs is dependent on the reaction conditions during manufacture; however the product is always a mixture of congeners. The general structure is that of a biphenyl with chlorine substituted at any of the various positions around each benzene ring (Figure 1.1). Theoretically, this allows for the possibility of up to 209 individual PCB congeners.

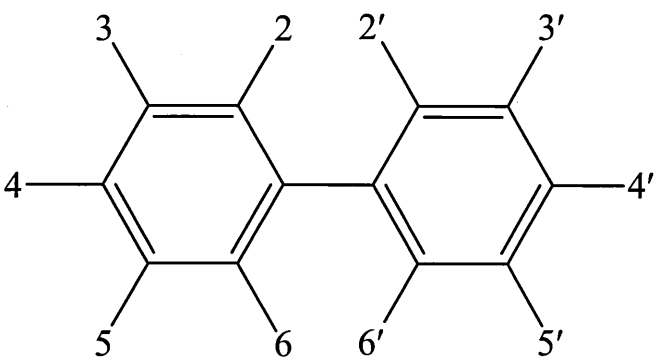

Figure 1.1 Generalised PCB structure.

Several systems of nomenclature have been employed for the identification of individual PCB congeners. Ballschmitter and Zell (1980) proposed a classification system, subsequently refined in 1992 (Ballschmitter et al., 1992), based on the number of chlorine atoms present, the biphenyl ring and the chlorine substitution pattern. In

addition they instigated a simple numbering system alongside the full nomenclature which numbers individual congeners in ascending order; for example 2,2′,5,5′-tetrachlorobiphenyl is also known as congener 52. The International Union of Pure and Applied Chemistry (IUPAC) subsequently adopted this system as the standard nomenclature (Table 1.1).

The phenyl rings of the PCB structure are able to rotate around the connecting bond and the preferred conformation is dependent on the pattern of chlorine substitution. Congeners with a chlorine atom present in the *ortho* positions (either 2,2′ or 6,6′) are often referred to as non-coplanar or non-planar PCBs, as this arrangement causes steric hindrance to molecular rotation. All other PCB congeners, i.e. those without *ortho* substitution are referred to as planar or coplanar PCBs, as the absence of chlorine at the *ortho* position allows rotation around the C–C bond of the biphenyl, enabling the congener to adopt a planar conformation. Non-*ortho*-substituted PCB congeners are particularly toxic as they have a biological mode of action similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as a result of their planar conformation (Walker, 2001). The relationships between conformation and toxicity are discussed further below (section 1.1.3).

1.1.2 PCB manufacture and use

As indicated in the previous section, the commercial manufacture of PCBs results in the formation of mixtures of different PCB congeners, the exact formulation depending on reaction conditions. PCBs were produced globally by a number of different manufacturers under a variety of trade names such as Aroclor, Pyroclor, Clophen, Phenochlor and Kanechlor. Monsanto was the major PCB manufacturer in the UK, producing the Aroclor series, one of the more commonly used ranges of PCB formulations.

Table 1.1 PCB congener nomenclature. Only those congeners analysed during this study are listed.

BZ number	IUPAC name
8	2,4′-dichlorobiphenyl
18	2,2′,5-trichlorobiphenyl
28	2,4,4′-trichlorobiphenyl
31	2,4′,5-trichlorobiphenyl
52	2,2,5,5′-tetrachlorobiphenyl
77	3,3′,4,4′-tetrachlorobiphenyl
101	2,2′,4,5,5′-pentachlorobiphenyl
118	2,3′,4,4′,5-pentachlorobiphenyl
126	3,3′,4,4′,5-pentachlorobiphenyl
128	2,2′,3,3′,4,4′-hexachlorobiphenyl
138	2,2′,3,4,4′,5′-hexachlorobiphenyl
149	2,2′,3,4′,5′,6-hexachlorobiphenyl
153	2,2′,4,4′,5,5′-hexachlorobiphenyl
169	3,3′,4,4′,5,5′-hexachlorobiphenyl
170	2,2′,3,3′,4,4′,5-heptachlorobiphenyl
180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl

In general, commercial mixtures are named according to their trade name and degree of chlorination. For example, the Aroclor series are named according to the number of carbon atoms present and the percentage weight of chlorine; hence Aroclor 1254 contains 54% chlorine. Various studies have characterised the congener composition of these technical mixtures (Frame et al., 1996). The typical proportions of individual congeners in some commonly used Aroclor mixtures are shown in Table 1.2.

Table 1.2 The proportions of PCB congeners occurring in several Aroclor mixtures (data taken from Frame et al. (1996)).

Congener BZ number	Aroclor 1242	Aroclor 1254	Aroclor 1260
8	7.00	0.05	0.04
18	8.53	0.08–0.25	0.05
28	6.86	0.06–0.19	0.03
31	7.35	0.11–0.28	0.04
52	3.53	0.83–5.38	0.24
77	0.31	0.03–0.20	ND
101	0.69	5.49–8.02	3.13
118	0.66	7.35–13.59	0.48
126	ND	0.02	ND
128	0.02	1.42–1.71	0.53
138	0.10	5.80–5.95	6.54
149	0.06	1.82–3.65	8.75
153	0.06	3.29–3.77	9.39
169	ND	ND	ND
170	ND	0.35–0.52	4.11
180	ND	0.42–0.67	11.38

PCBs are chemically inert and resistant to degradation. They have low vapour pressures, low electrical conductivity and are hydrophobic compounds with log *n*-octanol/water partition coefficients (log K_{ow}) of between 5.1 and 8.3 (Erickson, 2001). Physical and chemical properties vary across individual PCB congeners and are associated with the degree of chlorination. It is largely due to these properties that PCBs have been extensively used for a range of industrial applications, with the largest single use being as dielectric fluids in closed system transformers and capacitors. They have also been

widely utilised as hydraulic and heat transfer fluids (semi-closed systems), in lubricating oil formulations and as constituents in the manufacture of printing inks, adhesives, flame retardants and plastics (WHO, 1993). It is estimated that 1.3 million tonnes have been produced globally (Breivik et al., 2002a) since 1930.

PCBs were first identified as environmental contaminants during the mid 1960s (Jensen, 1966). As concerns over their environmental persistence and toxicity emerged, restrictions governing their production and use were progressively introduced from the early 1970s both in the USA and Europe. This process culminated in the United Nations Environmental Program (UNEP) Stockholm Convention on Persistent Organic Pollutants (POPs) (2001). This identified PCBs as one of 12 priority POPs which require global regulation as they are environmentally persistent, readily bioaccumulate and exhibit the potential for long range transport and adverse effects. In the UK the use of PCBs in all new equipment was prohibited from 1986 (Robinson and Lenn, 1994).

1.1.3 PCBs as environmental contaminants

Although their production and use has been stopped for two decades, PCBs are still released to the environment primarily from closed and nominally closed systems still in use. Continued release through disposal via landfill, incineration and open burning also contribute to current environmental releases (Breivik et al., 2002b). Environmental partitioning contributes to the movement and redistribution of previously released PCBs from contaminant sinks such as soil and sediment into the wider environment. These sinks account for much of the current PCB source (Harrad et al., 1994).

PCBs are highly persistent in the environment with half-lives varying from 70 days to several years between individual congeners (Beyer et al., 2000), within different environmental media and under different environmental conditions. Environmental persistence is typically determined by the mode of release (i.e. whether to soil, water or

atmosphere) and the degree to which the PCB partitions between these phases (Webster, 1998). Moreover, the extent to which a contaminant distributes between organic, aqueous and gaseous phases is driven by several physical properties namely vapour pressure, water solubility, *n*-octanol-water (K_{ow}), air-water (K_{aw}) and air-*n*-octanol (K_{ao}) partition coefficients (Beyer et al., 2002).

Long-range atmospheric transport of PCBs away from their site of release to remote areas such as the Arctic (Muir, 1999b) and the Canadian Great Lakes (Fox, 1998) has been well documented. Meijer et al. (2003) reported soil dry-weight PCB concentrations ranging from 26 pg g^{-1} to 97 ng g^{-1} from samples taken from rural sites world wide. Studies have shown there is a tendency for the PCB profiles of these areas to favour lower chlorinated congeners, thus providing, evidence that long range transport of PCBs occurs by means of global fractionation (Meijer et al., 2002), in that the more volatile lower chlorinated congeners enter the atmosphere in temperate regions and subsequently condense and are deposited in colder regions. Gouin et al. (2004) suggest that this mechanism occurs as a series of volatilisations and depositions from source rather than one single step – the 'grasshopper' effect – resulting in spatial variation in congener patterns.

The wide-scale, long-term use of PCBs coupled with their longevity in the environment and spatial distribution means PCBs have been detected in all environmental media (Nakata, 2005). Of particular concern is the tendency for these compounds to bioaccumulate (Kunisue et al., 2006; Leonards et al., 1998). As their K_{ow} values indicate, PCBs are lipophilic compounds and are readily sequestered into biological tissue to such a level that body concentrations are greater than PCB concentrations in the surrounding environment. The level to which a compound is bioconcentrated is determined by the rates of uptake and elimination. Uptake of PCB residues is primarily through dietary intake and adsorbtion across the gastrointestinal tract into the blood

stream. As a consequence of their lipohillic nature, PCBs are readily distributed into body tissues, in particular body fat. Additionally PCB residues within the blood stream undergo metabolism in the liver and subsequent elimination (Timbrell 1989). The level to which different PCB congeners are sequestered into body tissues is influenced by the susceptibility of individual congeners to metaolism, hence congeners which are readily metabolised have lower tissue concentrations than those congeners which are resistant to metabolism (Walker 1990). The process of uptake, distribution and elimination of PCBs within the body is a dynamic system with the distribution of PCBs between body tissues and the blood in equilibrium (Figure 1.2).

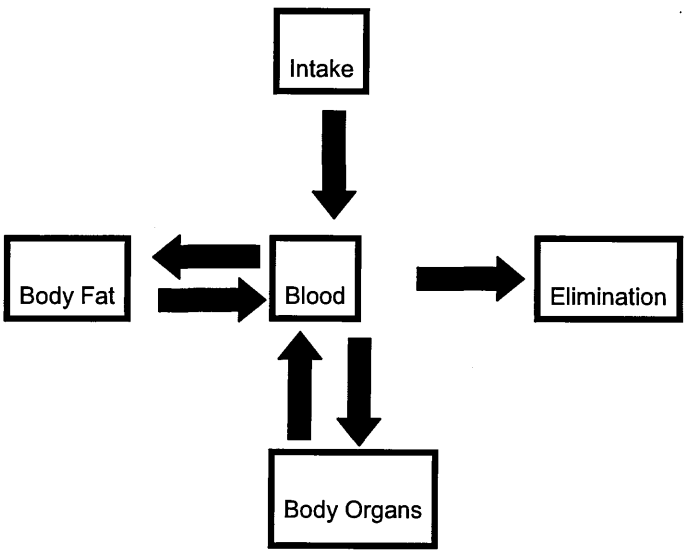

Figure 1.2 Distribution and dynamics of PCB residues within the body.

In addition to bioconcentration within individuals, PCB concentrations in biota increase as they are transferred up trophic levels of a food chain resulting in significant biomagnification (Johnson-Restrepo et al., 2005; Morrisey et al., 2005; Harari et al., 2004; Falandysz et al., 2002; Muir et al., 1999). The biomagnification of persistent organic pollutants like PCBs is primarily influenced by species and individual differences in the capacity to bio-transform and eliminate these compounds (Fraser et al., 2002). For PCBs, this results in inter-species variations in the congener profiles determined within a food chain (Falndysz, 2002; Trowbridge and Swackhamer, 2002;

Kannan et al., 2001; Boon et al., 1989) and between organisms of similar trophic levels across different feeding habits (Sagerup et al., 2002; Hoshi et al., 1998) as some congeners are more readily metabolised than others (Borga and Wolkers, 2005; Fraser et al., 2002; Miyamoto and Klein, 1998) and species vary in their capacity to metabolise PCBs (Ronis and Walker, 1985; Walker, 1990).

1.1.4 Adverse effects of PCBs

The exposure to PCBs has been associated with a wide range of harmful effects in vertebrates. Toxicological responses include impaired reproduction, immuno-toxicity, developmental deformities, endocrine disruption, carcinogenicity, neurological damage and hepatotoxicity (Fisher et al., 2000; Tilson and Kodavanti, 1998; Grasman et al., 1998, 2001; Ludwig et al., 1996; Harper et al., 1993; Safe et al., 1993, 1994).

The toxicity of individual congeners is dependent on their structure (Safe et al., 1985). Non-*ortho*-substituted PCBs are particularly toxic as their planar conformation enables them to bind to the aryl hydrocarbon receptor (A*h* receptor) on cytoplasmic protein. Additionally a group of mono-*ortho*-substituted PCBs (PCB BZ numbers 105, 114, 118, 123, 156, 157, 167 and 189) also exhibit A*h* receptor affinity, albeit lower than that of the non-*ortho*-substituted PCBs. Interaction with the A*h* receptor results in the induction of hepatic cytochrome P450 enzymes (Walker, 1998) and is the mechanism that underlies many of the observed toxicological effects of PCBs.

To assist the risk assessment of PCB and dioxin exposure the toxic equivalent factor (TEF) approach was developed (Ahlborg et al., 1994). This concept relates the toxicity of various non-*ortho* and mono-*ortho*-substituted PCBs to the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is given a reference TEF value of 1 (Table 1.3). As shown in Table 1.3, the potencies of individual congeners vary by orders of magnitude and differ between mammals, fish and birds.

*Table 1.3 Toxic equivalent factors associated with non-*ortho *and mono-*ortho *substituted PCB congeners. Values are taken from Ahlborg et al. (1994).*

PCB BZ number	Toxic Equivalent Factor		
	Mammals	Fish	Birds
77	0.0001	0.0001	0.05
81	0.0001	0.0005	0.1
105	0.0001	< 0.000005	0.0001
114	0.0005	< 0.000005	0.0001
118	0.0001	< 0.000005	0.00001
123	0.0001	< 0.000005	0.00001
126	0.1	0.005	0.1
156	0.0005	< 0.000005	0.0001
157	0.0005	< 0.000005	0.0001
167	0.00001	< 0.000005	0.00001
169	0.01	0.00005	0.001
189	0.0001	< 0.000005	0.00001

Toxic effects associated with exposure to *ortho*-substituted PCB congeners have also been reported (DeHaan et al., 1996). The effects are thought not to involve the A*h* receptor although precise mechanisms are not known. Di-*ortho*-substituted PCBs have been shown to interfere with estrogen and androgen receptor activity (Bonefeld-Jorgensen et al., 2001) and impair neurological development by interfering with calcium regulation in the brain (Tilson and Kodovaki, 1997, 1998; Seegal, 1991).

1.2 Environmental monitoring of PCBs

1.2.1 Why is contaminant monitoring of PCBs required?

As discussed in the previous section, PCBs are environmentally significant because of their ubiquity, persistence and toxicity. If we are to manage the risks posed by this

presence it is important to know the magnitude of environmental levels of PCB residues and to understand how they change over time. Because PCBs are not uniformly distributed, but are concentrated along pathways, characterised by different sources and dispersal mechanisms, the spatial patterns of these residues are also important. Therefore, monitoring of PCB residues is needed for the following purposes.

1. To assess ambient PCB concentrations in different environmental compartments and how they vary spatially and over time. The temporal and spatial aspect of contaminant monitoring provides baseline information of current contaminant levels and can provide an effective method for identifying the impact of human activities (White and Geitner, 1996).

2. To elucidate transport and fate pathways and thus understand the mechanisms of PCB behaviour within the environment.

3. To assess exposure of an organism to PCBs either through the direct measurement of PCB concentrations in tissue or indirectly through extrapolation from soil, sediment, air or food/prey concentrations (Olafsdottir et al., 2001; Gutleb and Kranz, 1998). Because PCBs are ubiquitous and persistent and concentrations are spatially variable, exposure is likely to be chronic and variable.

4. To assess biological response. Contaminant data obtained through regular monitoring is readily linked to studies on biological and ecological responses to PCB exposure and can provide valuable evidence of the likely impacts on exposed individuals and populations. This has been shown to particular effect by the various studies carried out correlating increased PCB concentrations to biological responses, typically liver enzyme responses (Kuyz et al., 2003), embryo toxicity and congenital deformities (Ryckman et al., 1998; Ludwig et al., 1996) and adverse reproductive

effects in bird species from the Great Lakes (Grasman et al., 1998, Nisbet et al., 1998; Ewins et al., 1992).

5. To provide environmentally realistic data for the development and validation of transport, fate and risk models (Hickie et al., 2005). Thus, monitoring data is valuable not only in providing information on current and historical situations but is equally valuable in the prediction of future scenarios. Further to this, PCBs can be used as model compounds to predict fate and behaviour characteristics of similar less documented pollutants.

6. Ultimately this knowledge is required to allow informed policy development and management strategies for limiting PCB exposure in the wider environment. Monitoring programs provide an effective mechanism for assessing the success of control and mitigation measures brought in to reduce PCB levels at local, national and global scales. Of particular relevance is the recognition by UNEP of PCBs as priority chemicals requiring global regulation as part of the Stockholm Convention on POPs 2001. Governments signed up to the treaty are required to put in place measures to reduce or eliminate PCB stocks and emissions; continued global monitoring of PCBs has been identified as key to evaluating the effectiveness of the convention.

1.2.2 A review of temporal and spatial trends in PCB concentrations

On the whole, PCB concentrations have declined with time since monitoring studies began during the 1970s. The extent to which PCBs have decreased varies both with the environmental compartment studied and spatially. In general, the rate at which PCBs have declined has slowed over the last ten to fifteen years. The magnitude of PCB concentrations remain little changed in remote areas due to the continued redistribution of these compounds from source regions. Aguilar et al. (2002) highlighted the

importance of continued monitoring in these regions as they are considered potential sinks for PCBs and other POPs now and in the future.

Long-term atmospheric monitoring of PCBs has generally shown decreasing PCB concentrations over the past decade (Sun et al., 2006; Hung et al., 2005; Jaward et al., 2004; Simcik et al., 1999; Hillery et al., 1997). Hung et al. (2005) have shown that the rate of decline in concentration can vary between individual congeners. Ambient PCB concentrations also vary seasonally and are influenced by changes in temperature which can affect the rate of volatilisation and air/surface exchange (Gouin et al., 2005; Ma et al., 2004; Halsall et al., 1999). Congener profiles vary with latitude as the effect of global fractionation results in higher proportions of lower chlorinated PCBs with increasing latitude (Jaward et al., 2004; Hung et al., 2001).

Analysis of archived soils from the UK shows increasing PCB concentrations from the 1950s up to the early 1970s; PCB concentrations declined significantly thereafter (Lead et al., 1997).

Contaminant monitoring in wildlife has been extensive as birds and mammals are often used as indicators of general environmental concentrations and trends. Temporal trend data covering the last 30 years are available for PCB concentrations in a number of different avian and mammal species.

PCB residue data obtained for wildlife from the Great Lakes region generally show significant declines in PCB concentrations in eggs (Hughes et al., 1998; Hebert et al., 1998; Ryckman et al., 1998), fish (Harrison et al., 2006; Suns et al., 1993), and other biota (Nisbet et al., 1998) over the last 30 years. Typically the rate of these declines has slowed during the last decade with PCB concentrations showing little change in recent years. Interestingly, PCB concentrations of herring gull (*Larus agrentatus*) livers monitored over a similar period show no evidence of decreasing (Fox et al., 1998).

Braune et al. (2001) measured PCB residues in kittiwake (*Risa tridactyla*), thick-billed murre (*Uria lonvia*) and fulmar (*Fulmarus glacialis*) eggs between 1975 and 1998 from the Canadian Arctic. Total PCB concentrations declined with time but further studies (Braune and Simon, 2003) indicated congener differences in temporal behaviour with non-*ortho* PCB residues increasing in northern fulmars. PCBs in Arctic ringed seal and seabirds have undergone a similar pattern of decline to that observed in the Great Lakes biota in that concentrations fell rapidly between the 1970s and 1980s and levelled off in subsequent years. However, PCB residues in other Arctic marine mammals do not appear to be declining (Muir et al., 1999a). Similarly, PCBs in seabird eggs and tissues from other areas have fallen over the last 30 years (Olafsdottir et al., 2005; Haffner et al., 1997; Weseloh et al., 1997; Oxynos et al., 1993).

Monitoring data for marine mammals shows broad spatial differences in PCB concentrations with the lowest concentrations recorded in polar regions, whereas marine mammals from Europe and the USA had the highest levels of PCB contamination. Overall PCB residues in marine mammals have declined significantly in those areas which were heavily contaminated with PCBs whilst levels in mammals from remote regions have increased somewhat as a result of the redistribution and global transport of PCBs (Aguilar, 2002).

There is a limited number of long-term monitoring studies using terrestrial mammals. Nevertheless Danish otters collected over ten years from 1980 showed declining PCB tissue residues (Mason and Madsen, 1993). Shore et al. (2006) reviewed several national and regional studies of contaminants in UK otters carried out during the 1980s and 1990s. In general, individual PCB congeners declined during that time and the rates of decline were congener specific. They noted that, in contrast to other studies (Bradshaw et al., 2002), total PCB concentrations in otters from England and Wales did not change over time. In accord with other long-term studies on PCBs in wildlife, the

rate of decline in PCB concentrations measured in UK otters appear to have been less marked during the 1990s than in previous decades.

Predatory birds have been widely used as indicator species for PCB contaminants and the reasons for this will be discussed further in section 1.3. Declines in PCB concentrations have been reported by a number of studies monitoring PCB concentrations in raptor and heron eggs over the past 30 years (Scharenberg and Looft, 2004; Elliott et al., 2001; Nygard, 1999; Weber et al., 1998; Newton et al., 1989, 1991, 1999). However, the declines in PCB concentrations appear to have levelled during the 1990s (Scharenberg, 2004; Elliott, 2000; 2001). This is consistent with declines in PCB concentrations in wildlife as previously discussed. In contrast to data collected for eggs, PCB concentrations do not appear to have declined in the tissues of raptor species (Shore et al., 2005; Kenntner et al., 2003a; Wegner et al., 2005; Johnstone et al., 1996). Spatial differences in PCB concentrations in both the eggs and tissues of predatory birds have been reported. Generally, the spatial variation in PCB residues was correlated with urban and industrialised areas (Wegner et al., 2002; Elliott et al., 2001; Nygard and Gjershoug, 2001; Elliott et al., 2000; Wieymeyer et al., 1993).

1.3 Using predatory birds to monitor PCB residues

PCB concentrations have been monitored in the eggs and tissues of predatory birds since the early 1970s (Shore et al 2005; Wegner et al., 2002; Nygard et al., 1999) for several reasons as outlined below.

1. Predatory birds feed at the highest trophic levels of their food chains and have been shown to be exposed to high levels of PCBs as a consequence of the biomagnification of PCB concentrations up through their food chains (Drouillard et al., 2001). Following exposure PCBs are readily bioaccumulated by predatory birds and partition into organs, blood system and fat reserves within the body (Walker, 1990). The high magnitude of

exposure and subsequent accumulation of PCB concentrations pose a toxicological risk to predatory birds.

2. PCBs have been associated with toxic and adverse population effects in various predatory bird species such as impaired reproduction (Elliott et al., 2001; Fernie et al., 2001; Grasman et al., 1998; Ryckman et al., 1998), physiological development (Harris et al., 2005; Fernie et al., 2003; Ryckman et al., 1998), and immuno-toxicity (Smits and Bortolotti, 2001; Barron et al., 1995).

3. Predatory birds are likely to be particularly sensitive to adverse ecological effects of PCBs and other contaminants. Although PCBs are not acutely toxic to individuals, any overall effect on reproductive success is likely to impact on population sizes. Predatory birds tend to have relatively small populations therefore any factor causing a reduction in the population will have a detrimental affect on population sustainability.

4. Predatory birds are specialist feeders and can therefore represent different food chains. Differences in congener profiles and concentrations between predatory bird species have been attributed to dietary differences (Herzke et al., 2003; Senthilkumar et al., 2002; Lopez-Lopez et al., 2001; Henny et al., 1998). Predatory birds can therefore provide an indication of the type of contaminants that biomagnify through a food chain. Predators are also used as indicator species because they accumulate higher levels of a contaminant than organisms of lower trophic levels and therefore detection of contaminants by analytical methods is more likely.

5. Predatory birds can act as sentinels for wider environmental changes in the effects of contaminants (Fox, 2001). Population effects may be detected sooner in these species given their smaller population sizes in comparison to other species.

PCBs are readily transferred to eggs as they are highly lipophilic and this is one route of elimination in female birds (Bargar et al., 2001). Therefore, eggs are commonly used to

monitor temporal and spatial trends in PCB concentrations as they provide a relatively consistent matrix, free from many influences that may mask the detection of trends, in that they are representative of a subsection of the population i.e. breeding females. In contrast, PCB residue data collected from the contaminant monitoring of biological tissues may be subject to differences in age, sex and various physiological factors such as individual metabolic capacity, starvation and disease; all of which have been shown to influence tissue PCB concentrations (Kenntner et al., 2003b; Donaldson and Braune, 1999; Anthony et al., 1993; Elliott and Shutt, 1993; Newton et al., 1981; Wieymeyer et al., 1989). These factors introduce considerable variability in tissue PCB concentrations between individuals and may well obscure environmental trends in these compounds. However, tissue residue data provide information on the overall population exposure that cannot be obtained through monitoring eggs alone and have been used to detect temporal changes in other organic lipophilic pollutants (Newton et al., 1993).

1.4 The Predatory Bird Monitoring Scheme

The Predatory Bird Monitoring Scheme (PBMS) is a long-term programme set up to monitor the levels of various pollutants in several British predatory bird species. The programme has been ongoing for over 35 years and is one of the longest running of its kind in the world. The scheme was initially set up to investigate the levels of organochlorine (OC) pesticides, such as DDT and dieldrin, and mercury-based fungicides in predatory birds following concerns over the effects of these chemicals on wildlife (Cooke et al., 1979). PCBs were included in the suite of compounds routinely analysed, following their confirmation as environmental contaminants by Jensen (1966). Since 1983 the exposure of barn owls (*Tyto alba*) and more recently kestrels (*Falco*

tinnunculus) and red kites (*Milvus milvus*) to second generation anticoagulant rodenticides has been included in the scope of the programme.

The scheme routinely monitors OC pesticide and PCB concentrations in the eggs and tissues of several different predatory bird species including seabirds. Thus it provides information on contaminant exposure and the risk posed to top predators representing different habitats namely, terrestrial, freshwater and marine and also differing food-chains. Eggs collected from merlin (*Falco columbarius*), golden eagle (*Aquila chrysaetos*), sea eagle (*Haliaeetus albicilla*) and gannet (*Morus bassanus*) are routinely analysed and in past years peregrine (*Falco peregrinus*) and sparrowhawk (*Acipiter nicus*) eggs have also been included in the scheme. Contaminant residues are also monitored in liver samples obtained from eurasian sparrowhawk, grey heron (*Ardea cinerea*) and until 1998 the common kestrel (*Falco tinnunculus*).

Bird carcasses are collected serendipitously from across the UK by volunteer collectors; indeed the scheme relies largely upon members of the public to send in carcasses they find in their locale. The birds collected will have died from a variety of causes; collision and starvation are amongst the two most commonly recorded (Newtom et al., 1999b). Consequently, birds of varying physiological conditions are included for residue analysis. All birds of the core monitoring species are analysed regardless of age, sex or cause of death.

The scheme has benefited from links to population ecology studies which have supplemented the contaminant data collected. This has enabled the scheme to demonstrate the adverse effects of organochlorine pesticides and PCBs on British raptor populations (Ratcliffe, 1970; Cooke et al., 1976; Newton, 1979) during the early years of the programme. The results of this work contributed to the introduction of restrictions imposed on OC pesticide and PCB use in the UK. Subsequently, the scheme has monitored levels of these contaminants to gauge the success of these bans by

determining whether concentrations have declined following mitigation. The data collected through the scheme have shown the decrease in organochlorine pesticides following restrictions and a corresponding recovery of raptor populations (Newton and Haas, 1984; Newton et al., 1992; Newton et al., 1993).

The unique spatial distribution of the samples collected allows data on contaminant levels to be obtained at both the national and regional scales. This spatial aspect of the scheme has been exploited to examine the influence of agricultural land use on liver HEOD and DDE concentrations in sparrowhawks and kestrels over the first 25 years of the program (Newton et al., 1993). Most recently the spatial distribution of liver PCB concentrations in sparrowhawks and kestrels has been investigated to determine whether hotspots of contamination could be identified using the data collected through the scheme (Broughton et al., 2003).

To date the PBMS has monitored total PCB residues in both eggs and livers of predatory bird species since the mid 1960s. The scheme has shown that PCB concentrations have declined in merlin and gannet eggs and also in heron livers. PCB residues in sparrowhawk livers have decreased slowly, whilst liver PCB concentrations in kestrels have remained at levels similar to those measured when monitoring began (Shore et al., 2005). In general PCB residues were higher in sparrowhawks and herons than in kestrels (Shore et al., 2005; Newton et al., 1993). Liver PCB concentrations are highly variable between individual birds and factors which may influence liver residue magnitude have not previously been accounted for when making species comparisons or assessing temporal trends and spatial variation in the data.

1.5 Aims and objectives

The overall aim of this thesis is to investigate and explain the main causes of intra- and inter-species variation in PCB residues in the livers of representative predatory birds, to

better inform our interpretation of wildlife contaminant data for PCBs and similar lipophilic compounds. Three species that are typiclly resident in the U.K. were selected for study, namely sparrowhawk, common kestrel and the grey heron; each representing a different food chain.

The sparrowhawk is widely distributed throughout the U.K and commonly occurs in deciduous and coniferous woodlands, farmlands and urban areas such as parks and gardens (Newton 1986). Its diet consists largely of small birds (Newton 1986). There are an estimated 40,500 breeding pairs in the U.K (BTO 2007) with a typical life expectancy of approximatly 3 years although a maximum recorded lifespan of upto 11 years has been reported (RSPB 2007). Breeding occurs after the first year with egg laying from the end of april and continuing through may.

The common kestrel is similarly resident throughout the U.K in a wide variety of habitats, typically farmland,open grasslands, heath, scrub and urban areas. Individual home ranges can vary upto 10 km^2 and depend largely on prey density, kestrel population density and season (Village 1990). Kestrels feed predominantly on small mammals with voles (*Microtus agrestis*) making up the majority of the small mammal prey. During periods when voles are scarce, kestrels switch to a wide variety of alternative prey species including invetrebrates, reptiles and amphibians and also small birds (Village 1990). Currently the BTO (2007) reports an estimated 37,000 breeding pairs in the U.K. with egg laying occuring between april and may. As with the sparrowhawk, average life expectancy is generally several years however lifespans upto 15years have been reported (Village 1990).

Grey herons are large (approx 1-2 kg) wading birds that occur in a variety of aquatic habitats across the U.K, feeding on a range of prey species including fish, amphibians and occasionally small mammals. Typical life spans are on average 5 years. There are

approximatly 14,000 breeding pairs in the U.K. with breeding occuring from february through to may (BTO 2007).

As described in section 1.1.3, the PCB congener profile determined in an individual is the product of spatial differences in PCB exposure, biotransformation through the food chain and individual capacity to metabolise PCBs. The influence of physiological condition, age or sex of an individual on the pattern of congener contamination in liver from free-ranging birds has not been documented.

This work draws upon the unique record provided by the PBMS and, in particular, on the results of analysis for 16 individual PCB congeners in the livers of sparrowhawk, kestrel and heron collected between 1992 and 2003.

The specific objectives of this thesis are outlined below.

6. To identify the major sources of intra-species variation in liver PCB concentrations in sparrowhawks, kestrels and herons and determine how these factors influence the magnitude of the PCB residues measured in liver.

7. To report for the first time, individual PCB congener concentrations for British sparrowhawks, herons and kestrels and determine typical PCB congener profiles for these three species.

8. To determine how total PCB and PCB congener concentrations vary between three predatory bird species representing different food chains and determine whether PCB congener patterns vary between the species.

9. To report Toxic Equivalent (TEQ) burdens for each species based on residue data for congeners 77, 118, 126 and 169 and compare TEQ burdens within and between species.

10. To re-evaluate historical temporal PCB data for sparrowhawks, kestrels and herons in light of the results obtained from intra-species analysis of residue variation. This will confirm whether temporal trends are masked by physiological factors.

Chapter Two

Methods

2.1 Sample collection

All the samples analysed for this study were collected as part of the Predatory Bird Monitoring Scheme (PBMS) carried out at the Centre for Ecology and Hydrology (CEH). Sample collection is an opportunistic process which relies on members of the public sending in predatory bird carcasses that they have found and the scheme regularly publicises the need for volunteer collectors through the wildlife press, poster presentations at conferences and public events, and the internet. A total of 405 sparrowhawks, 218 kestrels and 49 herons were received between 1992 and 2003 and are included in this study. These carcasses were sent in from across the United Kingdom (excluding Northern Ireland). Figures 2.1–2.3 (see overleaf) illustrate the geographical spread of the samples analysed for this investigation. On receipt, each bird was allocated a unique identification number and any accompanying information recorded. The location where each carcass was found was assigned to the nearest 10 km^2 OS grid reference and recorded as a four figure grid reference code. Carcasses were frozen at –20 °C until post-mortem examinations were carried out.

2.1.1 Provenance data recording

Post-mortem examinations were carried out on each carcass by a dedicated CEH staff member to ensure long-term continuity of the data recorded as part of the PBMS.

Figure 2.1 Distribution map showing the locations of sparrowhawk carcasses received by the PBMS between 1992 and 2003.

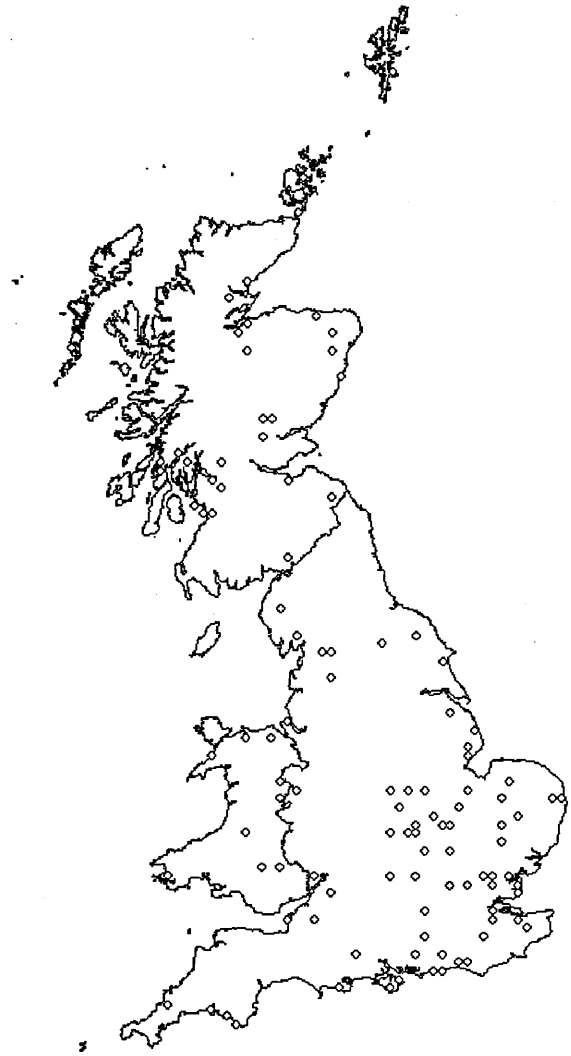

Figure 2.2 Distribution map showing the locations of kestrel carcasses received by the PBMS between 1992 and 1998.

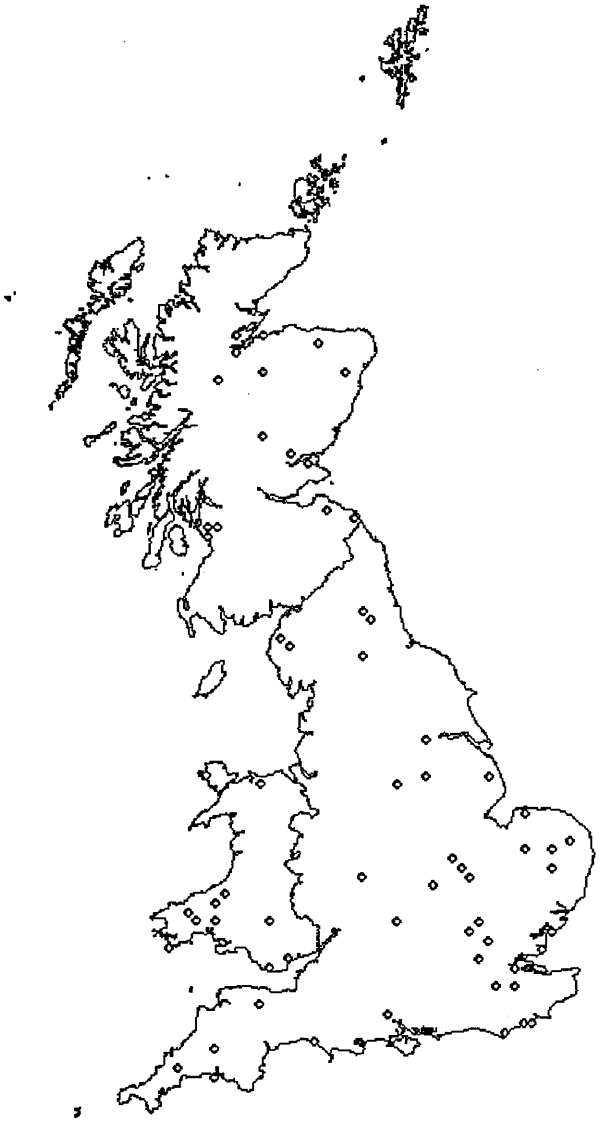

Figure 2.3 Distribution map showing the locations of heron carcasses received by the PBMS between 1992 and 2003.

Cause of death

The most likely cause of death was determined by visual inspection and allocated to one of sixteen classes (Table 2.1).

Table 2.1 Cause of death categories assigned to predatory bird carcasses collected as part of the PBMS.

Abridged cause of death descriptor	Abridged cause of death code	Extended cause of death descriptor	Extended cause of death code
Unknown	0	Unknown	1
		Unknown Trauma	6
Starvation & Disease	1	Starvation	7
		Disease	9
Poison	2	Poison	10
Accident	3	Window Collision	2
		Collision	4
		Starvation through injury	5
		Trauma	8
		Road Traffic Accident	11
		Human Predation	12
		Animal Predation	13
		Drowned	14
		Electrocution	16
Shot and Special Collection.	4	Shot	3
		Experimental Collection	15
		Euthanasia	15

Age

The age of each bird was categorised using the European Union for Bird Ringing (EURING) age categories as detailed in Table 2.2. Allocation to each category was based upon an individual's plumage, moult pattern, and the size of a bird's bursa of Fabricius. The bursa of Fabricius is a pouch on the wall of the cloaca. The bursa is enlarged and more defined in young birds and is therefore an indicator of age. For PBMS recording purposes these classes were combined to form three groups, namely adult (score 0), juvenile (score 1) or undetermined.

Table 2.2 EURING and PBMS age classifications

EURING code	EURING description	PBMS age class	PBMS age code
0	Age unknown	Undetermined	
1	Pullus (bird in nest)	Juvenile	0
1J	Young bird left the nest but unable to fly	Juvenile	0
2	Fully grown year of hatching	Juvenile	0
3	Hatched during calendar year	Juvenile	0
4	Hatched before calendar year – exact year unknown	Adult	1
5.	Hatched during previous calendar year	Juvenile	0
6	Hatched before last calendar year – exact year unknown	Adult	1
7	Hatched 2 years ago	Adult	1
8	Hatched 3 or more years ago	Adult	1

Sex

Sex was determined by examining the bird's size (not used in starved birds), plumage and, where the condition of the bird allowed, gonads. The sex of each individual was recorded as 0 for male birds and 1 for female birds.

Body condition

From 1992 onwards the nutritional state of each bird was assessed by visual inspection of fatty tissue deposits associated with the thorax, abdomen and heart. Body condition was recorded using a nominal six-point scoring system on a scale of 0–5, where 0 = no observed body fat, 1 = trace (fat deposits still present around heart), 2 = small (fat deposits apparent on surface of the pectoral muscle), 3 = moderate (larger fat deposits on surface of pectoral muscle and some deposits around clavicle), 4 = good body fat (as with 3 but with additional deposits in abdominal cavity) and 5 = abundant body fat reserves. Birds scoring either 0 or 1 were considered to be in a state of starvation.

Physiological parameters

Total body weights and, where the condition of the carcass allowed, individual organ weights were recorded. Liver, kidney, brain, muscle and fat tissues were removed and archived at –20 °C.

2.2 Chemical analysis

Birds were analysed for PCB residues during the year following their collection and at random. Liver samples were taken for analysis and were assigned to batches of 15 unknown tissues with a unique batch and sample number allocated to each. These reference numbers were cross-referenced to the bird identification number and related to a single tissue sample and analytical procedure at a single point in time. This allowed for repeat analysis of a tissue and analysis of different tissues from the same individual

whilst maintaining the traceability of each sample throughout the analysis and reporting procedure.

2.2.1 Preparation and sub-sampling

Samples were extracted using hexane and acetone (HPLC grade, Rathburn Chemicals Ltd, Walkerburn, UK.).

Acid-washed sand and sodium sulphate (Analar grade VWR Merck, Lutterworth, UK.) were both heated at 700 °C for 5 hours in a muffle furnace to remove possible organic contaminants. Aluminium oxide (Analar grade, Sigma Aldrich, Poole, UK.) was similarly heated at 800 °C. This was then deactivated by adding 5% w/w deionised water and mixing thoroughly for one hour.

COSHH assessments were carried out on all reagents and PCB calibration standards used, in compliance with health and safety legislation. All analytical procedures were covered by a safe system of work and corresponding risk assessment as required by CEH health and safety policy. Procedures involving the use of organic solvents were carried out in NERC class 1 fume cupboards. Waste solvents and chemicals were collected and disposed of via a specialist waste disposal company.

All cleaned glass items were rinsed three times with a 50:50 mixture of hexane/acetone to reduce the potential risk of cross contamination with PCB residues present within the laboratory environment.

Approximately 1 g liver tissue was sampled into a pre-weighed 100 ml glass beaker. This was reweighed using a four place analytical balance (Mettler Toledo) and the sample weight calculated. This sub-sample was taken through the solvent extraction process. A further sub-sample of up to 1 g was weighed into a glass boiling tube and

dried at 80 °C for 24 hours. The dry liver sample was reweighed and the moisture content of the tissue determined gravimetrically.

2.2.2 Solvent extraction and cleanup

PCB residues were extracted from the liver tissue using a cold solvent extraction method as outlined by Shore et al. (2001). Liver tissue was ground up using approximately 20 g acid-washed sand to form a homogenous paste. Up to 10 g anhydrous sodium sulphate was added as a chemical drying agent and the mixture stirred until it was of free flowing consistency. A 30 ml aliquot of a hexane/acetone (50:50) mixture was added to each sample; these were then covered and left to soak for a minimum of one hour with occasional stirring.

The solvent layer was transferred to a 50 ml glass measuring cylinder and a further 10 ml aliquot of hexane/acetone added to the homogenised tissue, mixed and left to soak for 15 minutes. The solvent layer was again transferred to the measuring cylinder and the process repeated until 50 ml extract had been collected in the measuring cylinder. The cylinder was sealed, mixed thoroughly by inversion and left to settle for 12 hours.

A 25 ml aliquot of the extract was quantitatively transferred to a pre-weighed 30 ml glass universal vial. It was left to stand in a fume cupboard until the solvent had evaporated completely and was then stored in a desiccator for 12 hours to ensure the lipid residues were moisture free. The universal vials were weighed and the weight of solvent-extractable lipid calculated gravimetrically.

The lipid residues were reconstituted by re-dissolving them into 5 ml hexane for a cleanup step to isolate PCB residues from the interfering lipid compounds.

Interferences were removed using open column alumina chromatography. Columns were prepared by placing a small plug of glass wool into the neck of a Pasteur pipette

and rinsing several times with hexane. A total of 0.8 g of 5% deactivated aluminium oxide was added to each column and packed by gentle agitation to remove any air pockets.

A 1 ml aliquot of the reconstituted extract was transferred to the column and washed under gravity with 1 ml aliquots of hexane until a total of 5 ml had been collected. Dichlobenil (50 µl of a 5 µg ml^{-1} solution) was added to the cleaned-up extract as an internal standard.

2.2.3 Instrument parameters

PCB residues were quantified using capillary gas chromatography with electron capture detection. A Varian 3400 gas chromatograph equipped with a Varian 8035 liquid autosampler was used. Data acquisition was controlled by PC using EZCHROM software (Scientific Services, Cambridge).

A 4 µl sample of the cleaned-up sample extract was injected at 200 °C using a split/splitless injection technique. The split valve was programmed to be closed on injection and opened after 2 minutes. At 10 minutes into the analysis the split valve was closed in preparation for the next sample. The split flow was set to 50 ml min^{-1}. The autosampler needle and tubing were washed with acetone and hexane rinses between each injection to prevent contaminant carry-over between samples.

High-purity hydrogen and ECD grade nitrogen (Air Liquide) were used as the carrier and make-up gas respectively.

Between 1992 and 1996, chromatographic separations were carried out using two 30 m DB210 columns of 0.25 mm id and 0.25 mm film thickness (Jones Chromatography Ltd, Hengoed) connected in series. The carrier gas pressure was set to 20 psi to give a calculated flow rate of 35 cm s^{-1}. The column oven was programmed with a ramped

temperature program (Table 2.3). The detector temperature was set to 300 °C (Jacobs et al., 1997).

Table 2.3 GC oven parameters used for PCB analysis conducted between 1992 and 1996.

Temperature °C	Heating rate °C min^{-1}	Hold time min
90	NA	1
185	2.5	20
210	5	12
240	40	0

From 1996 onwards, the analysis was carried out using a 50 m HT8 column of dimensions 0.25 mm id and 0.22 mm film thickness. This was connected in series to a 5 m inert silica retention gap of 0.25 mm id. Carrier gas pressure was adjusted to 15 psi to give a calculated flow rate of 30 cm s^{-1}. The oven temperature program was adjusted as detailed in Table 2.4. The detector temperature was set at 320 °C (Shore et al., 2001).

Table 2.4 GC oven parameters used for PCB analysis conducted after 1996.

Temperature °C	Heating rate °C min^{-1}	Hold time min
50	NA	2
170	45	2.5
200	2.5	5
280	2	0
320	10	10

2.2.4 Calibration standards

Calibration standards were supplied as individual congener solutions of 100 μg ml^{-1}, in hexane (ChemService, via Greyhound Chemicals Ltd, Birkenhead).

A mixed sub-master standard containing all the PCB congeners, each at a concentration of 2 μg ml^{-1}, was made up by quantitatively transferring 2 ml of each congener solution to a single 100 ml glass volumetric flask. Once all congeners had been added, the volume was adjusted to 100 ml with hexane and the flask weight recorded.

Working calibration standards were made up by dilution from the PCB sub-master standard as indicated in Table 2.5. All standards were made up to volume with hexane.

Table 2.5 Dilutions and the resulting concentrations used to prepare calibration standards for PCB quantification.

Calibration level	Standard concentration required/ng ml^{-1}	Standard used for dilution/μg ml^{-1}	Volume used/ml	Final volume of working standard/ml
1	0.016	0.016	0.05	50
2	0.032	0.016	0.05	25
3	0.08	0.016	0.25	50
4	0.16	0.016	0.25	25
5	0.32	0.016	1.0	50
6	1.0	2.0	0.05	100
7	2.0	2.0	0.05	50
8	4.0	2.0	0.1	50
9	8.0	2.0	0.2	50
10	12.0	2.0	0.3	50

All standards were weighed on preparation. They were then weighed prior to any subsequent use and any loss in volume through evaporation of hexane during storage

was corrected by adding hexane until the recorded weight was achieved. Standards were re-weighed after each use and the weights recorded.

When new standards were prepared they were analysed against the existing standards and peak responses compared to ensure they had been prepared correctly.

2.2.5 Quantification and congener identification

PCB congeners were quantified using an internal standard calibration technique to account for the slight variations in injection volume which are inherent in any gas chromatography analysis. Dichlobenil was added to all working calibration standards and samples to give a concentration of 0.05 $\mu g\ ml^{-1}$. The ratio of congener peak area to dichlobenil peak area was used when calculating response factors for each congener to normalise any differences in injection volume between the standards and samples. Sixteen individual PCB congeners (IUPAC numbers 8, 18, 28, 31, 52, 77, 101, 118, 126, 128, 138, 149, 153, 169, 170 and 180) were quantified. A full 10 point multi-level calibration covering the concentration range 0.016 $ng\ ml^{-1}$ to 12 $ng\ ml^{-1}$ was analysed prior to each year's sample analysis and subsequently after every fourth batch of unknown samples during that analysis period. This allowed a routine check of ECD detector linearity, instrument sensitivity and calculation of response factors for each compound. The resulting linear regression equation for each congener was used to calculate concentrations in the unknown samples, with standard concentration as the predictor variable and peak area ratio the response variable.

A single working standard was analysed with every batch of unknown samples to check for inter-batch drift in the retention times of each PCB congener and also check that the response factors for each compound were as expected.

An instrument blank consisting only of hexane was analysed prior to each batch to ensure the GC system was clear from contaminants which might affect the sample analysis.

Data were acquired using the EZCHROM software. Peaks were identified by comparison of their retention time (relative to that of the internal standard) with those of each congener in the calibration standard. Unknown peaks falling within a predetermined time window for any particular congener were assigned as that congener. This was done automatically by the EZCHROM software. However; each chromatogram was checked manually against the standard chromatogram to verify peak assignment and integration. Peak data and instrument concentrations were exported to Microsoft Excel to calculate final wet weight concentrations. These calculations accounted for each dilution and concentration step within the extraction and cleanup procedures.

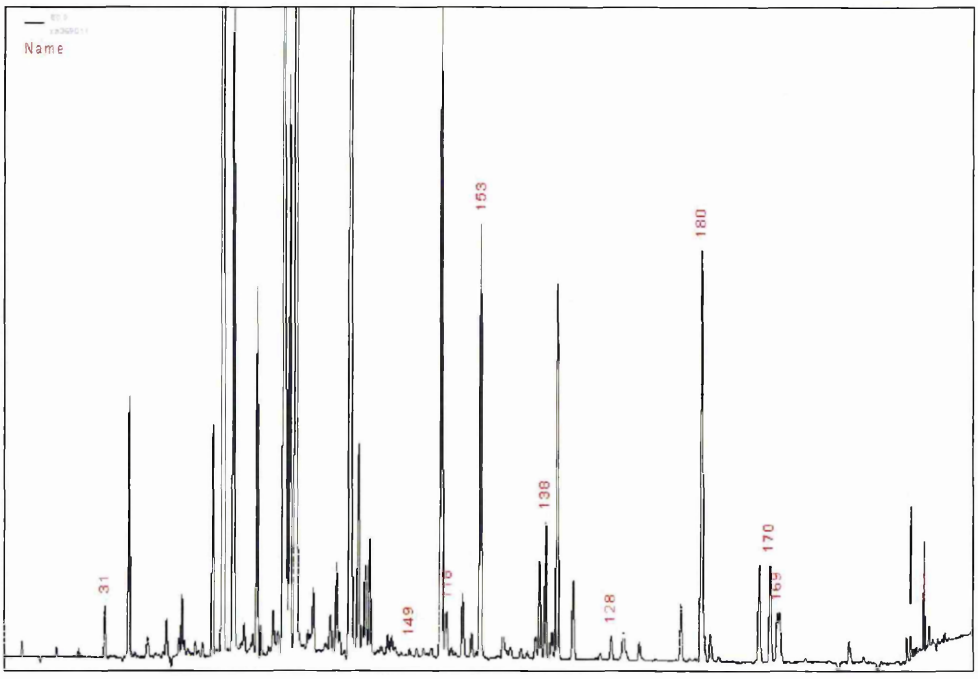

Figure 2.4 Gas chromatograph trace showing PCB residues quantified in a sparrowhawk liver.

2.2.6 Quality assurance

Quality assurance samples were extracted and analysed with each batch of unknown samples.

Analytical blank This contained only the reagents used during the extraction and cleanup. It was analysed to identify any possible contamination from either the reagents or the general laboratory environment during the sample preparation procedures.

Spike recovery sample A sample of clean chicken liver was spiked prior to extraction with 50 µl of the sub-master standard, to give a spike of 0.1 µg of each PCB congener in the extract. It was analysed to provide a similar sample matrix match to the unknowns to allow an assessment of the efficiency of the sample preparation stages and also identify possible interferences resulting from the liver tissue matrix. Results from analysis of the recovery sample were expressed as a percentage of the expected congener concentrations.

Control liver A replicate sample of clean chicken liver was analysed as a control for the spiked recovery sample. Recovery sample concentrations were corrected for any PCB congeners detected in the control sample before assessing the percentage recovery.

In-house tissue reference An in-house reference liver tissue containing several organochlorine pesticides was analysed using the same extraction and cleanup and quantification procedures outlined earlier. This homogenised tissue was analysed at regular intervals together with the bird of prey livers to provide replicate analysis throughout the monitoring period and hence an indication of the analytical consistency over time. Results falling outside a pre-determined 95% confidence interval for each pesticide were re-analysed along with all associated unknown samples.

Additionaly, a certified reference material CARP-2 (NRC) was analysed at regular intervals during the study period. This reference material was not analysed in

conjunction with the bird of prey samples but formed part of the wider routine quality assurance procedures within the Monks Wood laboratory.

2.2.7 Expression of results

All samples were corrected for any congeners detected in the analytical blank associated with their analytical batch. Liver PCB concentrations are expressed as $\mu g\ g^{-1}$ wet weight unless specified.

Limits of detection (LOD) were calculated for individual PCB congeners on a batch by batch basis. Prior to 1996, LODs were quantified using a peak response equivalent to three times the detector baseline noise (Miller and Miller, 1993). From 1996 onwards LODs were calculated from a subset of the standards run as part of the multilevel calibration. This method was based on the commonly used LOD definition of the mean response + three standard deviations of a series of instrument blanks. LODs were calculated from the regression equation of the lower five calibration standards (Zorn, 1997), ensuring the concentration range did not exceed one order of magnitude, using the following formula:

$$LOD\ response = Y_0 + 3\ S_{Y/X}$$

where Y_0 was the y intercept and $S_{Y/X}$ the standard error (SE) of the calibration regression (Miller and Miller, 1993).

Any sample concentration falling below the calculated limit of detection was recorded as a non-detected (ND) value. Mean ± SE limits of detection for the whole dataset are given in Table 2.6.

Table 2.6 Limits of detection for bird of prey liver samples analysed between 1992 and 2003.

PCB congener	Mean ± SE LOD/ ng ml^{-1}	N
8	0.68 ± 0.06	74
18	0.89 ± 0.08	74
28	0.34 ± 0.03	74
31	0.43 ± 0.04	74
52	0.67 ± 0.06	74
77	0.74 ± 0.14	74
101	0.31 ± 0.03	74
118	0.23 ± 0.03	74
126	0.24 ± 0.03	74
128	0.18 ± 0.02	74
138	0.23 ± 0.02	74
149	0.31 ± 0.03	74
153	0.34 ± 0.04	74
169	0.17 ± 0.02	74
170	0.14 ± 0.02	74
180	0.15 ± 0.02	74

Results were not corrected for percentage recovery; any results falling outside the range 60% to 110% were investigated to identify the error in analysis and whether the poor recovery result was indicative of problems in the whole analytical batch. Where this was the case the whole batch was reanalysed. Due to problems retrieving archived data, recovery data are only available for analytical batches analysed from 1997 onwards. Mean ± SE percent recovery data for this subset of data are given in Table 2.7.

Table 2.7 Percent recovery data for spiked chicken liver samples analysed between 1997 and 2003.

PCB congener	Mean ±SE % recovery	N
8	75 ± 4	26
18	93 ± 5	26
28	88 ± 5	26
31	87 ± 3	26
52	92 ± 4	26
77	92 ± 3	26
101	104 ± 4	26
118	94 ± 3	26
126	92 ± 3	26
128	96 ± 3	26
138	96 ± 3	26
149	93 ± 4	26
153	96 ± 3	26
169	91 ± 5	26
170	94 ± 5	26
180	91 ± 4	26

2.3 Data analysis

The data collected for each congener were checked for normal distribution prior to data analysis. In common with many environmental contaminant datasets, the liver PCB residues were not normally distributed as tested using an Anderson-Darling test for normality (sparrowhawk: AD = 44.49; kestrel: AD = 46.57; heron: AD = 5.86; $p < 0.005$ for all). Each species data were positively skewed (sparrowhawk: skewness =4.30, kurtosis = 26.75; kestrel: skewness =2.89, kurtosis = 9.14; heron skewness =9.71, kurtosis = 108.05). Therefore in order for each dataset to fulfil the requirements of parametric statistical tests, data were normalised by $\log_{10}$ transformation.

Individual sample limits of detection for each compound were generated by working up the instrument LOD for each congener using the concentration factors resulting from the sample preparation steps, and then normalising the resulting amount for sample weight to give an LOD expressed as $\mu g\ g^{-1}$ for each sample. Usually a single LOD, based on the mean sample weight is assigned to each non-detected result prior to data manipulation. However the sample weights varied significantly between species (one way ANOVA $F_{(2,563)} = 44.96$, $p < 0.001$), with heron liver samples approximately double the weight taken for analysis from either the sparrowhawk or kestrel livers. This difference resulted in highly variable sample LODs and taking a mean LOD across the species would result in a significant loss of concentration data from the overall dataset. In contrast, using the minimum LOD would artificially skew the data in favour of low congener concentrations. In order to allow transformation of the data and limit the effect of assigning values to non-detected results, a value of half the lowest measured concentration was assigned to non-detected results. This was done on a congener by congener basis (Table 2.8, overleaf).

Summary statistics have been given throughout as the geometric mean ± geometric standard error range. Statistical analysis was carried out using Minitab and Graphpad Prism version 4 software packages.

Table 2.8 Sample limits of detection for bird of prey samples analysed between 1992 and 2003.

Congener	Mean LOD ± SE/ng g^{-1}	Minimum LOD/ng g^{-1}	Maximum LOD/ng g^{-1}	Minimum measured concentration/ ng g^{-1}	N
8	30.0 ± 1.4	1.3	490.8	7.0	566
18	38.8 ± 1.8	1.6	556.4	28.0	566
28	12.7 ± 0.5	1.0	172.3	9.0	566
31	17.9 ± 0.9	0.6	318.8	2.0	566
52	27.5 ± 1.2	1.9	365.0	5.8	566
77	40.0 ± 3.1	0.4	755.7	4.0	566
101	12.3 ± 0.6	0.3	197.0	2.0	566
118	8.3 ± 0.4	0.3	118.2	1.0	566
126	9.1 ± 0.4	1.2	198.3	4.0	566
128	7.0 ± 0.4	0.4	148.9	1.0	566
138	8.3 ± 0.4	0.3	132.7	1.2	566
149	12.8 ± 0.6	0.3	219.3	5.0	566
153	12.4 ± 0.8	0.3	174.6	3.0	566
169	7.2 ± 0.5	0.2	214.1	0.4	566
170	5.2 ± 0.35	0.2	147.4	1.0	566
180	4.5 ± 0.3	0.2	93.4	1.0	566

Chapter Three

Identifying the sources of variation in liver PCB residues in birds of prey

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3.1 Introduction

The long term PCB data collected through the Predatory Bird Monitoring Scheme (PBMS) have previously shown that average PCB concentrations are generally higher in sparrowhawk and heron livers than in kestrel livers (Newton et al., 1993). The residue data for all three species are characterised by large variation between individuals, with the result that it can be difficult to detect and interpret differences between the species without accounting for other factors which may also influence liver PCB concentrations.

PCB residues in biological tissues are determined by a range of factors. In general, differences in contaminant levels between birds are largely attributed to location (Newton et al., 1993), dietary exposure (Hoshi et al., 1998; Newton et al., 1993) and the ability to metabolise organochlorine compounds (Fossi et al., 1995; Walker, 1990; Walker et al., 1987; Ronis and Walker, 1985). However age, sex and body condition can also significantly affect PCB concentrations in both tissues and eggs (Kenntner et al., 2003b; Donaldson and Braune, 1999; Anthony et al., 1993; Elliott and Shutt, 1993; Wieymeyer et al., 1989; Newton et al., 1981, 1992). Tissue PCB residues have been shown to rise following periods of starvation (Lambeck at al., 1991) and the increase is thought to be a result of remobilisation of persistent organic contaminants from fat depots. Residue levels also vary seasonally in association with fluctuations in body fat levels (Kenntner et al., 2003b; Olafsdottir et al., 1998; Subramanian et al., 1986). Although these studies have identified the individual factors which can affect tissue

PCB concentrations, the relative importance of these factors in explaining the variation in PCB residues between birds has not been determined.

The aim of the study described in this chapter was to explore the extent to which variation in total liver PCB concentrations in predatory birds collected through the PBMS is explained by differences between individuals in their age, sex, nutritional status and the season in which they died. The effect of each factor on residue magnitude in sparrowhawks, kestrels and herons was compared to determine whether their influence was similar across different species and how important they were when compared to the other factors which might account for both intra and inter-specific variation in PCB residues.

The results of this investigation were then used to inform a more detailed congener-specific analysis (Chapter 4).

3.2 Methods

In this chapter, liver PCB residues are expressed as $\mu g\ g^{-1}$ wet weight concentrations of the sum of the sixteen congeners that were determined ($\sum$congener PCB concentrations). For statistical purposes, samples in which no congeners were detected were assigned a nominal value of 0.003 $\mu g\ g^{-1}$, half the lowest congener specific limit of detection.

Routine monitoring of kestrel livers for PCB analysis by the PBMS was stopped after 1998. In order to ensure a consistent comparison between species, only the data held for sparrowhawks, herons and kestrels collected between 1992 and 1998 have been analysed for this study. Individual congeners were not determined before 1992 and there are no congener-specific data for birds that died in earlier years.

Liver PCB concentrations were first examined for temporal trends using linear regression. There was no significant time trend over the seven-year period for

sparrowhawks, kestrels or herons ($R^2 < 0.08$ in all cases). For each species therefore, the data for different years were pooled and treated as a single data set covering the entire period.

3.2.1 Intra-species variation in ∑PCB concentrations

A backwards, stepwise general linear model approach was used to determine the main factors influencing the variation in liver PCB concentrations; data for each species were analysed separately. Age, sex, body weight and fat score were entered as terms in an adjusted (type III) general linear model (GLM) along with their respective interaction terms; age, sex and body weight were treated as co-variables. This form of GLM computes the sum of squares for each term once all terms have been fitted in the model hence the outcome does not depend on the order in which terms are entered into the model as each is accounted for equally. Following analysis of the full model, the term that explained the least amount of variation and was not statistically significant was removed from the model. The analysis was then repeated continually until the only terms remaining were those that were statistically significant ($p < 0.05$).

3.2.2 Seasonal variation in ∑PCB concentrations

The sparrowhawk and kestrel datasets were examined for seasonal trends in separate analyses. The heron dataset was not analysed in this way as there were insufficient data to carry out a similar assessment. The seasonal analysis was carried out independently of the earlier analyses because sample numbers were too few to allow the earlier GLM models to run when season was incorporated as a term.

Birds were allocated to different seasons on the basis of the carcasses being found in one of four, 3-month periods. For the first-year birds, these periods were set so that the first quarter included the time when young become independent of their parents. The first-quarter periods were taken as August to October for sparrowhawks (Newton,

1986), and July to September for kestrels which hatch earlier (Village, 1990). For adult birds, the seasonal periods were set so as to follow egg laying and the first quarter was taken as June to August for both species (Newton, 1986; Village, 1990). Because of the temporal differences in the quarterly periods for birds of different age, the data for first-year and adult birds were analysed separately. Season, fat score, sex and their interactions were entered as terms in the GLM.

3.2.3 Inter-species variation in ∑PCB concentrations

Inter-species differences in liver PCB concentrations were also examined using GLMs and data for first-year and adult birds were analysed separately. In each case, species was entered as a term in the model along with the other factors that had been identified as significant during the earlier analysis of intra-specific variation. To ensure there were adequate numbers of birds with different fat scores for all three species, individuals were assigned to one of only two body condition classes, namely starved (fat scores 0 and 1) and non-starved (fat scores 2–5).

3.3 Results

A total of 306 sparrowhawk, 186 kestrel and 47 heron carcasses were analysed. Congener sum PCB concentrations were detected in the livers of 90% of sparrowhawks and 88% of kestrels and in all but a single first-year female heron. The overall (including the birds with non-detectable residues) geometric mean (geometric standard error (GSE) range) wet weight concentrations in sparrowhawks were 0.342 (0.304–0.384) $\mu g\ g^{-1}$. Kestrels and herons had geometric mean concentrations of 0.013 (0.009–0.019) $\mu g\ g^{-1}$ and 0.645 (0.522-0.797) $\mu g\ g^{-1}$ respectively. In general, adults had higher liver PCB concentrations than first-year birds (Table 3.1), whilst both adult and first-year males had higher PCB residues than their female counterparts.

Table 3.1 Geometric mean (one geometric standard error range) liver PCB concentrations ($\mu g\ g^{-1}$ wet wt) in UK predatory birds during 1992–1998.

Species	Adult Male	Adult Female	First-year Male	First-year Female
Sparrowhawk	0.899 (0.732-1.102) n=45	0.704 (0.620-0.943) n=57	0.294 (0.240-0.361) n=88	0.193 (0.156-0.238) n=122
Kestrel	0.201 (0.140-0.287) n=33	0.036 (0.020-0.066) n=18	0.178 (0.139-0.228) n=41	0.140 (0.111-0.174) n=93
Heron	0.941 (0.186-4.756) n=3	0.851 (0.470-1.540) n=5	0.882 (0.648-1.200) n=22	0.394 (0.290-0.534) n=17

3.3.1 Intra-specific variation in liver PCB concentrations

In sparrowhawks, variation in liver PCB concentration was significantly explained by three factors. These were fat score ($F_{(1,275)} = 35.0$, $p < 0.001$), sex ($F_{(1,275)} = 8.23$, $p = 0.004$), and age ($F_{(1,275)} = 7.82$, $p = 0.006$). The interaction between the age and sex terms was also just significant ($F_{(1,275)} = 3.87$, $p = 0.05$). Body condition, as determined from fat score, was the dominant influence, accounting for some 37% of the variation in the magnitude of liver residues. PCB concentrations were highest in starved birds (fat score 0) and decreased progressively as the fat scores of birds increased (Figure 3.1). Overall, geometric mean liver PCB concentrations were 3 to 30 times higher in starved than in non-starved individuals. The effect of age was that liver residues were higher in older birds. Adults on average had liver PCB concentrations that were two to four times greater than those in first-years and this difference was consistent across birds in each fat class category (Figure 3.1). Although sex was a significant factor, the magnitude of

its effect differed with age, as indicated by the significant age*sex interaction term. In first-year birds, males always had higher liver PCB concentrations than females with the same fat score whereas, in adult birds, males and females had roughly similar liver PCB concentrations. Age and sex together accounted for a further 7% of the variation in liver PCB concentrations in sparrowhawks.

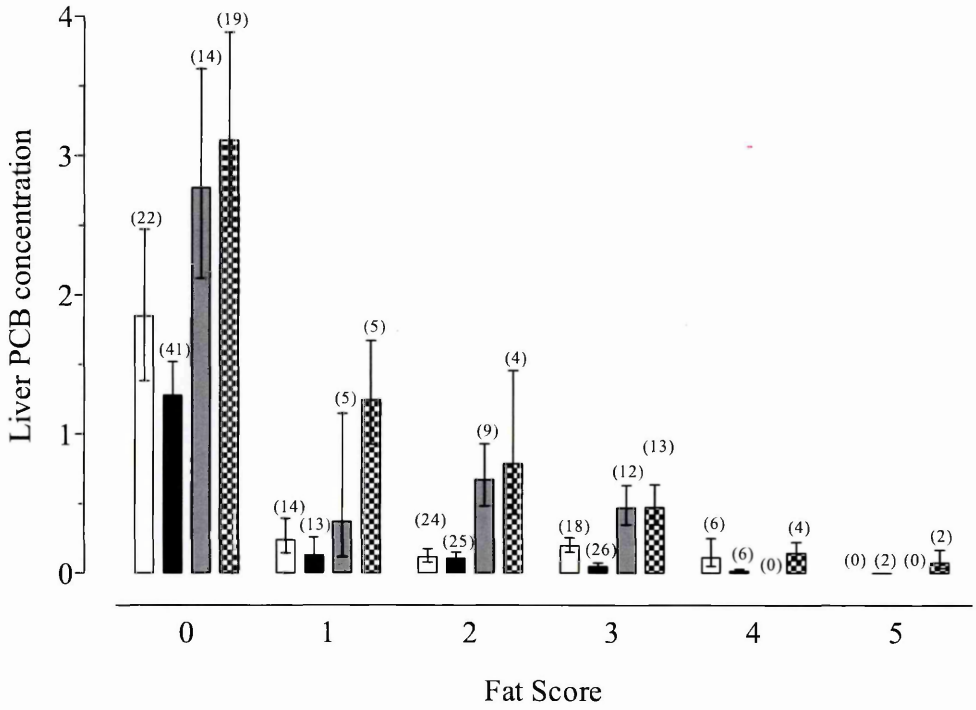

Figure 3.1 Geometric mean (and GSE range) liver congener sum PCB concentrations (μg g^{-1} wet wt) in sparrowhawks with different body fat scores. First-year male (open bars), first-year female (hatched bars), adult male (grey bars) and adult female (chequered bars). Sample sizes are indicated in parenthesis.

Amongst the kestrels that were analysed, one adult female had an unusually high liver PCB concentration (33.0 μg g^{-1}) that was outside three geometric standard deviations of the mean. This was considered an outlying result and was removed from the dataset as it had an undue influence on the statistical model that investigated seasonal variation in residue magnitude; its removal had no significant impact on the outcome of any of the other analyses. As with sparrowhawks, body condition was the most significant factor that influenced residue magnitude in kestrels ($F_{(1,169)} = 36.0$, $p < 0.001$). It explained

49% of the variation in liver PCB concentration and residues were generally lower in fatter birds (Figure 3.2). Sex was also a statistically significant factor ($F_{(1,169)} = 8.43$, $p = 0.004$) and accounted for 5% of the variation in PCB concentrations (Figure 3.2). Males generally had higher liver PCB concentrations than females but, unlike in sparrowhawks, there was no evidence that this trend differed significantly between first-year and adult birds, although it was somewhat more marked in adults (Figure 3.2). Age was also a statistically significant factor in the GLM ($F_{(1,169)} = 8.23$, $p = 0.005$) and, like sex, accounted for some 5% of the total variation in liver PCB concentrations. Residues were higher in adults than in first-year birds (Figure 3.2). This was not apparent from the overall mean liver PCB concentrations for adult and first-year kestrels (Table 3.1) because a high proportion (64%) of the first-year birds were starved and this masked the effect of age on residue magnitude.

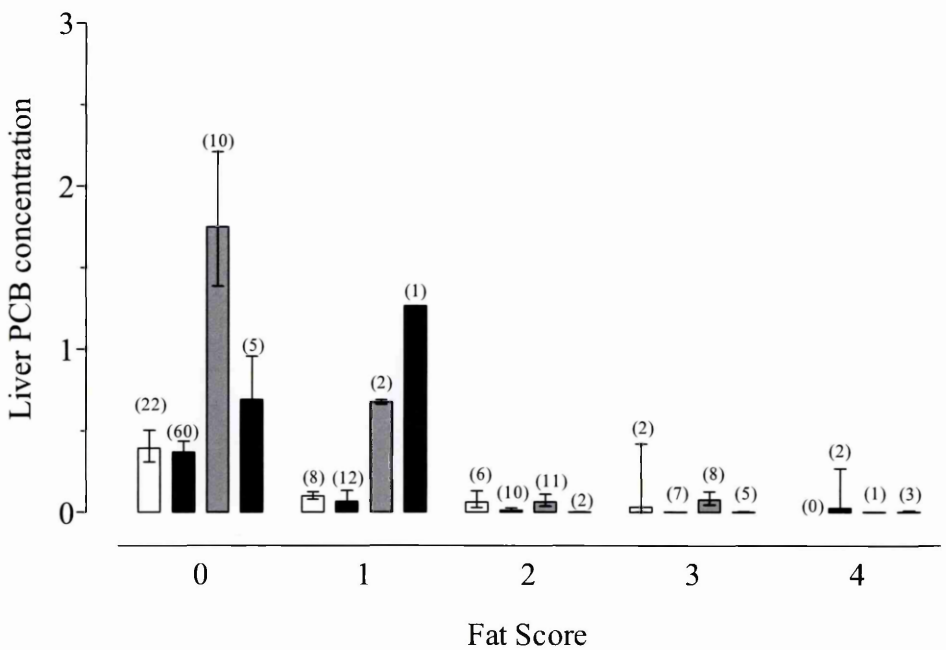

Figure 3.2 Geometric mean (and GSE range) liver congener sum PCB concentrations (μg g^{-1} wet wt) in kestrels with different body fat scores. First-year male (open bars), first-year female (hatched bars), adult male (grey bars) and adult female (chequered bars). Sample sizes are indicated in parenthesis.

Fat score was the only factor that significantly explained individual variation in liver PCB concentrations in herons ($F_{(1,39)} = 2.99$, $p = 0.02$). It accounted for 38% of the variation in liver PCB concentration. As with sparrowhawks and kestrels, liver residues were generally lower in fatter birds (Figure 3.3). There was no evidence that other factors such as age or sex significantly affected liver PCB residues, although the sample size was relatively low and the statistical power of the analysis may have been insufficient to detect such effects.

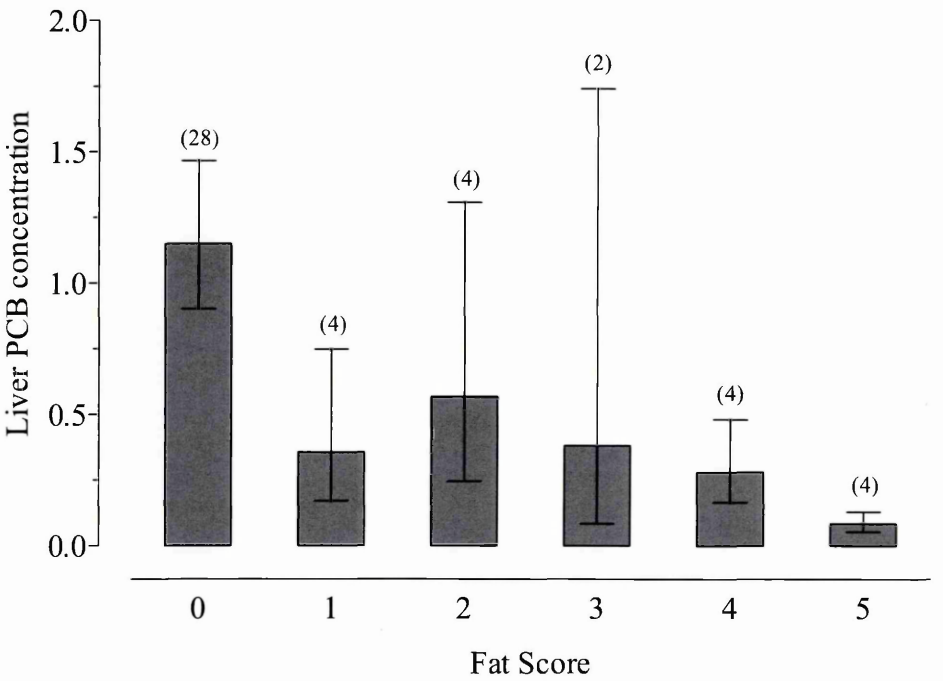

Figure 3.3 Geometric mean (and GSE range) liver congener sum PCB concentrations (μg g^{-1} wet wt) in herons with different body fat scores. First-year male (open bars), first-year female (hatched bars), adult male (grey bars) and adult female (chequered bars). Sample sizes are indicated in parenthesis.

3.3.2 Seasonal variation in ∑PCB concentrations

Liver PCB concentrations differed significantly between seasons in first-year sparrowhawks ($F_{(3,184)} = 13.1$, $p < 0.001$). Residues increased progressively between August and the following April (Figure 3.4) and reached a peak in the third quarter

of the year (February–April). However, PCB concentrations in birds that died between May and July (the final quarter) were on average lower than those in birds from the preceding months. This may have been because the May–July sample contained early-fledged birds from the current breeding season that had not been distinguished at post-mortem from year-old birds, and may also have included individuals that had bred in their first-year and excreted PCBs in their eggs. The seasonal pattern of accumulation

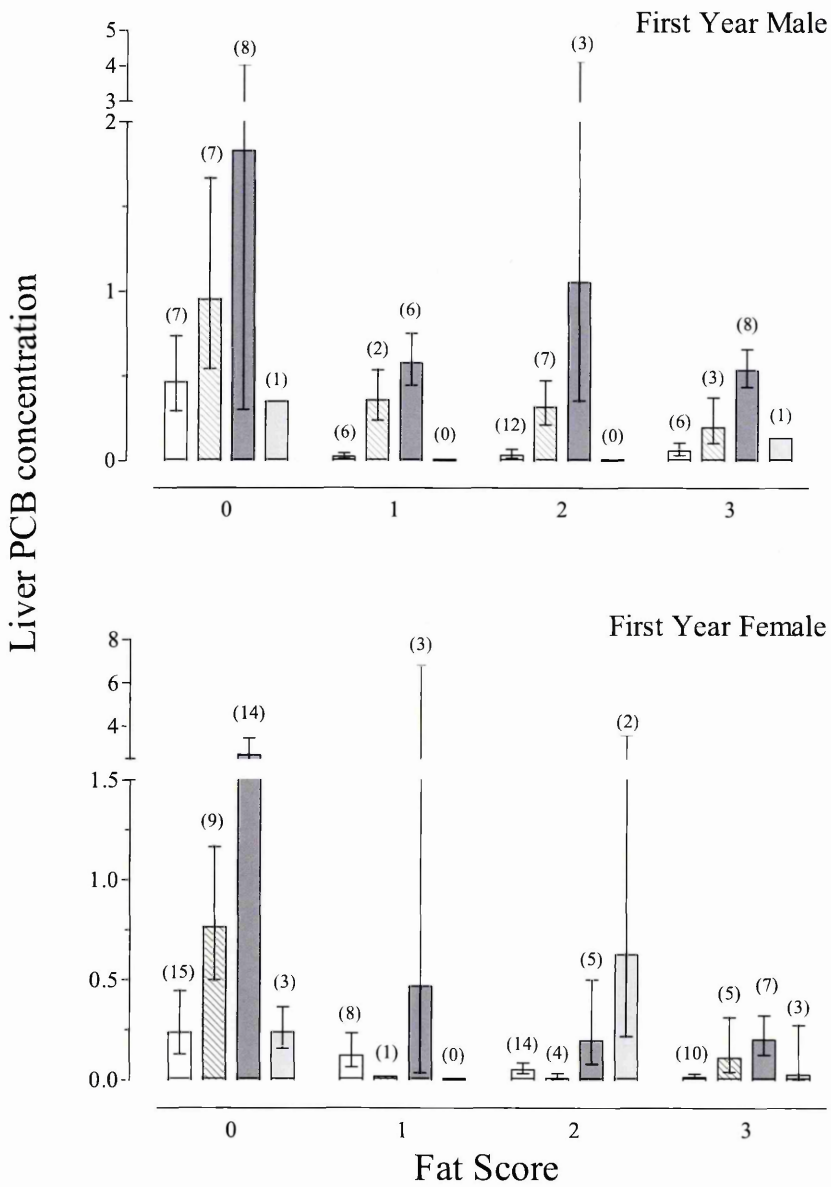

Figure 3.4 Geometric mean (and GSE range) liver sum PCB concentrations (μg g^{-1} wet wt) in first-year male and female sparrowhawk carcasses found during different quarters of the year. The periods were Aug–Oct (white bars), Nov–Jan (hatched bars), Feb–Apr (dark grey bars) and May–Jul (pale grey bars).

was similar for birds in each fat score category. As in the previous analysis, both fat score and sex were significant factors in the GLM for first-year sparrowhawks ($F > 5.53$, $p < 0.02$ in both cases). Within each three-month period, starved birds contained higher liver PCB concentrations than birds with higher fat scores, and males had higher PCB concentrations than females with the same fat score.

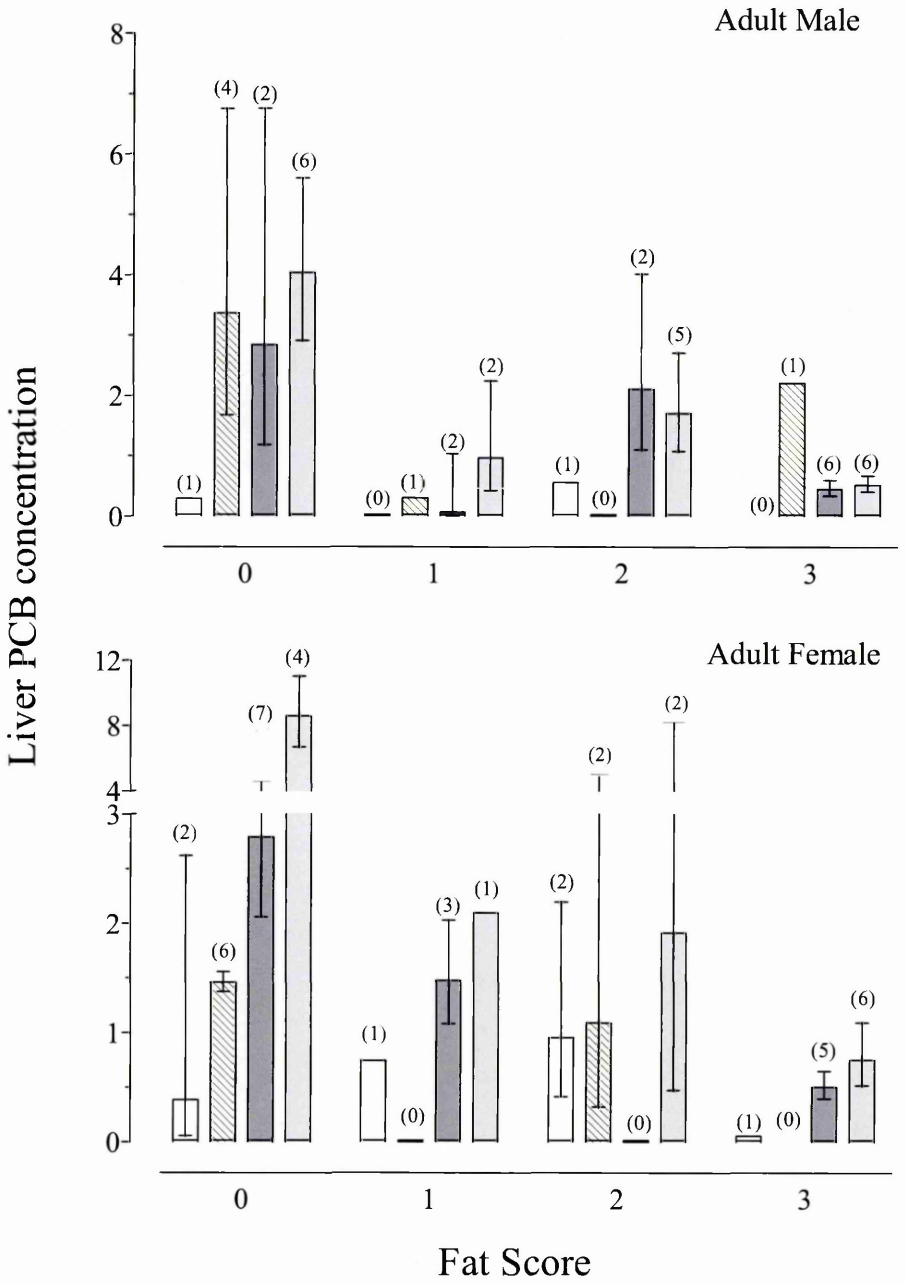

Figure 3.5 Geometric mean (and GSE range) liver sum PCB concentrations (μg g^{-1} wet wt) in adult male and female sparrowhawk carcasses found during different quarters of the year. The periods were Jun–Aug (white bars), Sept–Nov (hatched bars), Dec–Feb (dark grey bars) and Mar–May (pale grey bars). (See overleaf for discussion.)

Liver PCB concentrations did not vary significantly between seasons in adult sparrowhawks (Figure 3.5). Although there was some indication that liver PCB concentrations accumulated progressively in females during the year (Figure 3.5, bottom graph), neither the season nor sex/season interaction term were statistically significant. Fat score was the only significant term in the analysis ($F_{(5,81)} = 13.3$, $p < 0.001$).

Kestrels were similar to sparrowhawks in that there was significant seasonal variation in liver PCB concentrations in first-year ($F_{(3,121)} = 4.87$, $p = 0.003$, Figure 3.6), but not

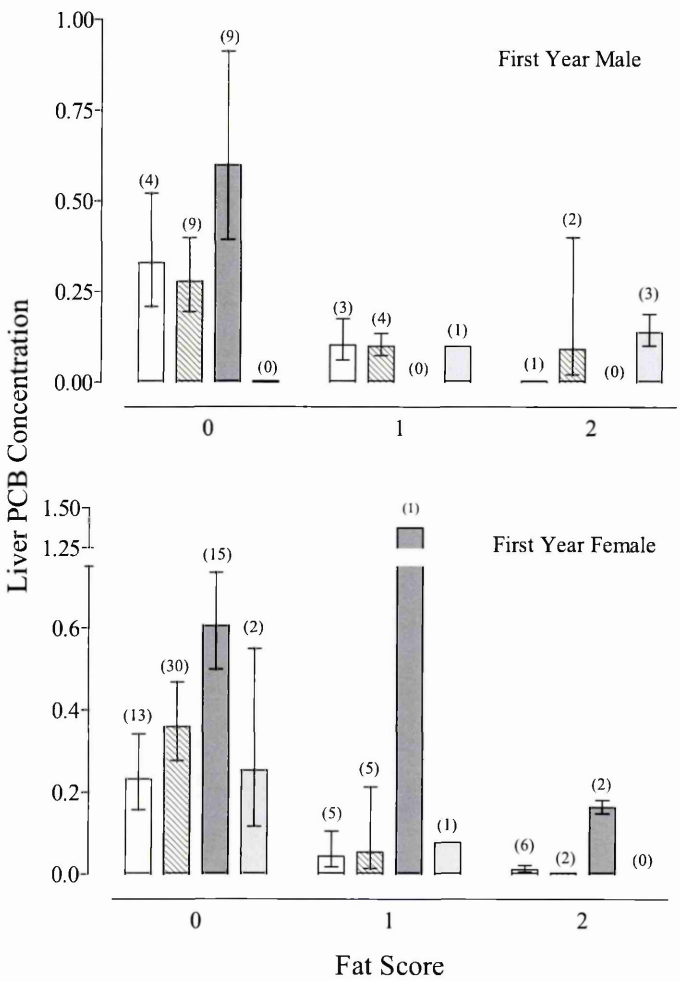

Figure 3.6 Geometric mean (and GSE range) liver sum PCB concentrations (μg g^{-1} wet wt) in first-year male and female kestrel carcasses found during four annual periods. Jul–Sept (white bars), Oct–Dec (hatched bars), Jan–Mar (dark grey bars) and Apr–Jun (pale grey bars).

adult (Figure 3.7) birds. Liver concentrations generally increased throughout the year in the first-years, although the pattern was not as distinct as that in first-year sparrowhawks. Fat score was a significant factor in the GLMs for both first-year and adult kestrels ($F \geq 24.4$, $p < 0.001$ in both cases) and sex was also a significant factor in adults ($F_{(1,42)} = 15.4$, $p < 0.001$), males having higher liver residues than females.

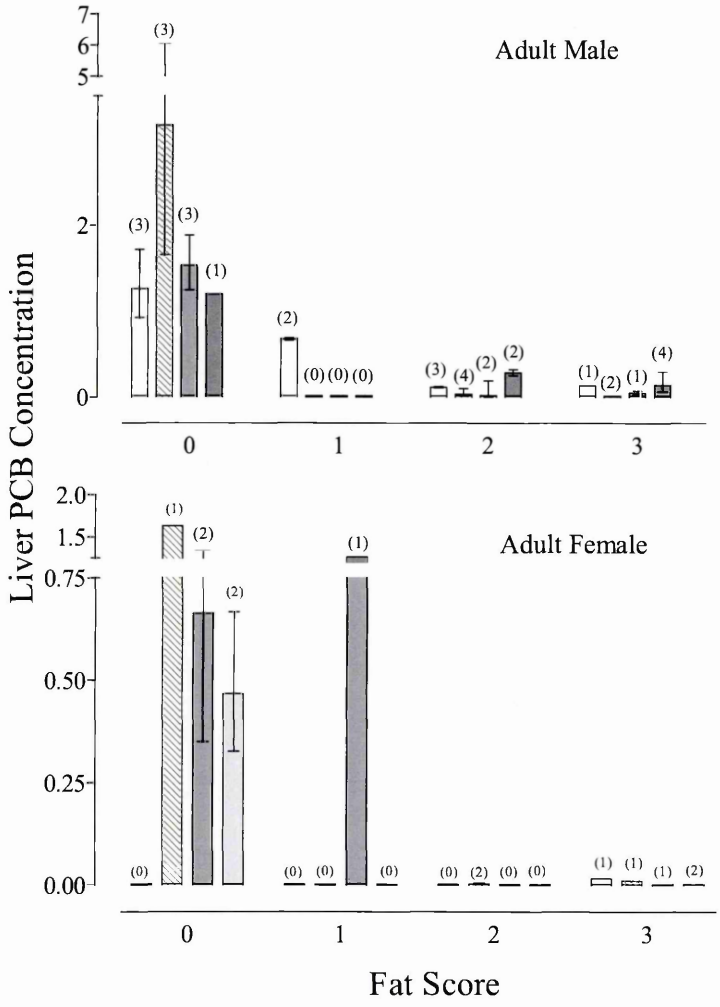

Figure 3.7 Geometric mean (and GSE range) liver sum PCB concentrations (μg g^{-1} wet wt) in adult male and female kestrel carcasses found during four annual periods. Jun–Aug (white bars), Sept–Nov (hatched bars), Dec–Feb (dark grey bars) and Mar–May (pale grey bars).

3.3.3 Inter-species variation in ∑PCB concentrations

Although the overall geometric mean liver PCB concentrations for all the birds that were analysed suggested that herons had the highest residues and kestrels the lowest (Table 3.1), these mean concentrations did not take into account the influence of fat

score, age, body weight or season. GLM analysis, which included fat score, sex and season as factors, confirmed there was significant variation between species in both first-year and adult birds ($F > 18.6$, $p < 0.001$ in both cases). In first-years, kestrels had significantly lower PCB liver residues than either sparrowhawks or kestrels (Tukeys *post-hoc* pairwise comparison tests: $t \geq 4.58$, $p < 0.001$ in both comparisons) and, as in previous analyses, residue magnitude varied with fat score, sex and season ($F > 6.70$, $p < 0.001$ in all cases). Liver PCB concentrations were higher in birds with low fat scores, increased progressively throughout the year, and were generally higher in males than females in each quarter of the year (Figure 3.8). In adult birds, kestrels again had lower liver PCB concentrations than sparrowhawks ($t = 6.09$, $p < 0.001$) but fat score had a major influence on this species difference (species*fat score interaction: $F_{(1,138)} = 6.52$, $p < 0.001$). Adult sparrowhawks, kestrels and herons in relatively poor condition (fat scores 0 and 1) had largely similar liver PCB concentrations (Figure 3.8).

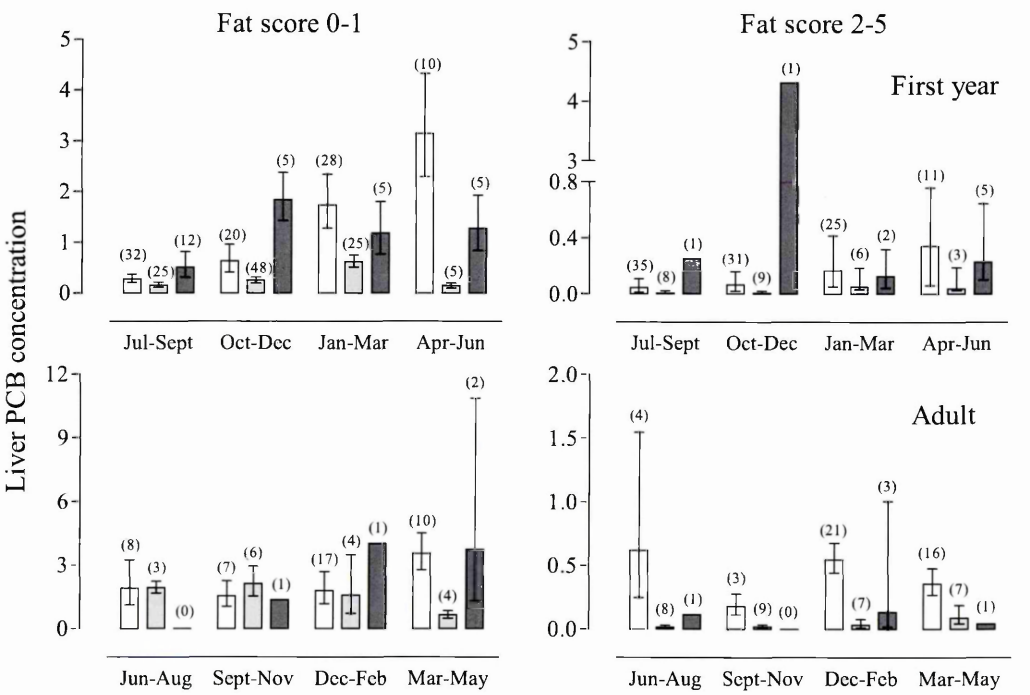

Figure 3.8 Geometric mean (and GSE range) liver sum PCB concentrations (μg g^{-1} wet wt) in different quarters of the year in first-year (top graphs) and adult (bottom graphs) sparrowhawks (white bars), kestrels (light grey bars), and herons (dark grey bars) with fat scores of 0–1 or 2–5. Sample sizes are indicated in parenthesis.

In contrast, sparrowhawks with higher (2–5) fat scores had liver residues that were an order of magnitude greater than in kestrels with similar levels of body fat. The liver PCB concentrations in herons with fat scores of 2–5 were generally lower than in sparrowhawks but higher than in kestrels, although the number of adult herons analysed was small. Overall, species differences accounted for 8% and 14% of the variation in liver residues in first-year and adult birds. This compared with 20% and 31% that was accounted for in first-year and adult birds by body condition, season and sex.

3.4 Discussion

The aim of the analysis described in this chapter was to identify the main sources of variation in the liver PCB concentrations of sparrowhawks, kestrels and herons in the UK during a period when annual mean liver concentrations did not increase or decrease progressively in any of the species.

The summary concentrations reported here are generally lower than those reported by other studies for raptor species analysed over a similar time span (Table 3.2).

The lower PCB concentrations reported in this study occur because only a limited number of PCB congeners were quantified and subsequently used to calculate a sum PCB concentration. As shown in Table 3.2, other studies used a variety of methods to quantify PCB concentrations (Aroclor-matched, Clophen-matched or total PCB basis) and therefore data from the present study are not directly comparable with liver concentrations given elsewhere. Nevertheless, the congeners quantified are ones which are typically found in predatory birds in Britain and largely span the range of chlorination and physico-chemical properties of all the PCB congeners. The PCB concentrations reported here can therefore be considered representative of total PCB burdens in the birds, and the factors that affected sum concentrations are expected to have identical effects on total PCB residues.

Table 3.2 Liver PCB concentrations reported in several predatory bird species.

Species	Mean PCB Concentration/ $\mu g\ g^{-1}$	PCB Quantification	Reference
Sparrowhawk	2.72 (0.37–19.96)	Total Arochlor 1254	Newton et al., 1993
Kestrel	1.93 (0.32–11.47)	Total Arochlor 1254	Newton et al., 1993
Heron	1.82 (0.18–18.19)	Total Arochlor 1254	Newton et al., 1993
Goshawk *Accipiter gentilis*	6.607 ± 13.94	$\sum$ 7 congeners	Kenntner et al., 2003b
White tailed Eagle *Haliaeetus albicilla*	0.375 ± 0.628 (juvenile) 9.02 ± 21.5 (adult)	$\sum$ 7 congeners	Kenntner et al., 2003a
Bald Eagle	0.429–3.36	$\sum$ 28 congeners	Elliott et al., 1996
Buzzard *Buteo buteo*	4.344	Arochlor 1260	Lopez-Lopez et al., 2001

The analysis demonstrated that a combination of nutritional state, age, sex, and time of death can account for much of the variation in liver PCB residues in predatory birds. Together, these factors were equally, or more, important in explaining variation in residues than both geographical variation in exposure or inter-species differences in detoxification capability. Body condition, as measured by fat score, was the single most important factor. The remobilisation of organochlorine compounds from depleting fat stores, and associated increased concentration of these compounds in body organs, has been reported previously (Kenntner et al., 2003b; Elliott et al., 1996; Newton et al., 1992; Lambeck et al., 1991; Subramanian et al., 1986; Cooke et al., 1979), but the relative importance of this process in determining the magnitude of liver residues was not quantified by these studies. In sparrowhawks, kestrels and herons that were analysed

for the present study, the liver PCB concentrations in birds with little or no body fat were up to 30-fold higher than in non-starved individuals of the same age and sex. Fat score alone explained between 37% and 49 % of the total intra-species variation in liver PCB concentration. This was despite birds being from widely dispersed locations that may have had markedly different environmental concentrations of PCBs.

Age also explained some of the variation in residue magnitude, and liver residues were typically higher in adult than first-year sparrowhawks and kestrels. Similar age-related differences in contamination have been reported for blood plasma (Johnstone et al., 1996; Elliott and Shutt, 1993; Wieymeyer et al., 1989), breast muscle (Olafsdottir et al., 1995) and liver (Kenntner et al., 2003b; Platteeuw et al., 1995) PCB concentrations in other predatory bird species. The higher liver residues in adult than in first-year sparrowhawks and kestrels, and in adult birds of other species, probably reflect the longer time-frame over which adults have been exposed to PCBs and a slow rate of elimination relative to uptake resulting in accumulation of PCB residues in the tissues. Differences in prey choice between first-year birds and adults may also impact on the level of exposure to PCBs. This may certainly be the case for juvenile kestrels which eat more invertebrates than adult kestrels (GilDelgado et al., 1995; Village, 1990).

The progressive increase in liver PCB concentrations in sparrowhawks and kestrels during their first year after fledging (Figures 3.4 and 3.6) is further evidence for age-related accumulation in these species. It was notable that the mean PCB concentrations reached at about the end of the first year of age were generally lower than the residues in adult birds. This suggests that sparrowhawks and kestrels may not accumulate their maximum PCB loads until after their first year and is consistent with the finding that PCB concentrations in the eggs of sparrowhawks only reached a maximum during the second breeding season (Newton et al., 1981). Organochlorine contaminant concentrations in osprey (*Pandion haliaetus*) eggs (Ewins et al., 1999) and in the blood

plasma of sharp-shinned hawks (*Accipiter striatus*) (Elliott and Shutt, 1993) likewise have been found to increase progressively with age, at least until the time of first breeding.

In comparison to sparrowhawks and kestrels, herons have relatively poor metabolic capacity to detoxify PCBs (Walker, 1990) and are prone to accumulating higher levels of PCBs and other organochlorine compounds. Age-related differences in liver PCB residues might also be expected in this and other *Ardeidae* species (Niethammer et al., 1984). Mean liver PCB concentrations were higher in adult than in first-year herons (Table 3.1) but the difference was not statistically significant. It is likely that the low number of adults in the sample restricted the power of the analysis to detect a significant age-related difference. It is also possible that dietary PCB exposure is higher in juvenile than adult herons as a result of different prey choice (Moser, 1985), and this offsets any differences in PCB accumulation related to length of exposure.

Sex was a third significant factor that influenced liver PCB residues in sparrowhawks and kestrels. Higher levels of PCB contamination in males than females have been reported in sharp-shinned hawks (Elliott and Shutt, 1993), American white pelicans (*Pelecanus erythrorhyncos*) (Donaldson and Braune, 1999) and various migratory species (Tanabe et al., 1998). Lower residues in females have generally been thought to be due to transfer of contaminants into eggs (Tanabe et al., 1998) and female sparrowhawks are thought to lose up to a third of their organochlorine contaminant body burden in this manner (Newton et al., 1981). This process may also account for reported seasonal variations in the differences between males and females in their organochlorine residue levels (Donaldson and Braune, 1999; Subramanian et al., 1986). The higher liver PCB concentrations in male than female adult kestrels in the present study may similarly be due, at least in part, to loss of PCBs to eggs. The apparent progressive increase in liver PCB residues in adult female sparrowhawks after the time

of egg laying (Figure 3.5) is also consistent with maternal transfer of PCBs into eggs and subsequent accumulation during the rest of the year. Overall, though, there was no significant difference in liver PCB concentrations between adult male and female sparrowhawks, nor were sex-related differences in PCB residues detected in a smaller sample of goshawks (*Accipiter gentilis*) from Germany (Kenntner et al., 2003b). It is possible that adult female sparrowhawks and goshawks, which are larger and take bigger prey than adult males (Newton, 1979), have higher PCB exposure levels than males and this offsets the annual loss of contaminants into eggs. However, neither contaminant transfer to eggs nor higher exposure of females can explain why liver residues were greater in male than female first-year sparrowhawks and kestrels. This suggests that other sex-related factors must also be important in determining liver PCB concentrations in these species.

When the liver PCB residues of sparrowhawks, kestrels and herons were compared and factors such as fat score, sex and age were taken into account, kestrels were still found to have the lowest liver PCB concentrations of the three species. This is likely to be partly due to differences in exposure related to the respective food chains of the three species (Newton et al., 1993). Compared with birds, mammals have higher activities of the cytochrome P450 enzyme systems involved in PCB metabolism (Ronis and Walker, 1989). Kestrels feed predominantly on small mammals (Village, 1990) and so may be exposed to lower dietary concentrations of PCBs than either sparrowhawks, which feed predominantly on birds, or herons which mostly eat fish. Furthermore, the small mammals on which kestrels feed are largely herbivorous and so may have only relatively low level exposure to PCBs themselves. In addition, kestrels have a higher hepatic P450 activity than sparrowhawks, and fish-eating birds have significantly lower P450 activity than most other avian species (Walker, 1990; Ronis and Walker, 1989; Walker et al., 1987). Thus, kestrels are likely to metabolise and excrete a greater

proportion of their ingested PCBs than either sparrowhawks or herons. However, the results showed that the extent of these species differences in liver residues varied significantly with nutritional state. Liver PCB concentrations in birds with high fat scores (2 and above) were much lower in kestrels than in sparrowhawks and herons but, in starved birds, all three species had similar liver residues. This suggests that the pharmacokinetics and partitioning of PCBs within the body must vary significantly between the three species. It is also possible that the PCB-liver binding sites in these species become saturated following rapid remobilisation of PCBs from fat stores, although much higher liver residues have been detected in other species that have been experimentally dosed (Hoffman et al., 1996; Marsili et al., 1996).

This study has demonstrated that physiological factors, namely body condition and, to a lesser extent, age and sex, can account for much of the variation in liver PCB residues in predatory birds. Body condition is entirely an intrinsic physiological factor and so it is perhaps not surprising that its effect on liver residues was similar in all of the species that were examined. In contrast, sex and age-related effects on residues may be mediated through differences in prey choice, food intakes per unit body weight, egg number, and other individual physiological characteristics, and so their influence on residue magnitude can be species-specific. Overall, these three physiological factors explained up to approximately half of intra-specific variation in liver PCB concentrations in sparrowhawks, kestrels and herons that were collected between 1992 and 1998.

Chapter Four

PCB congener profiles in predatory birds

4.1 Introduction

PCB concentrations in predatory birds reported by CEH's Predatory Bird Monitoring Scheme (PBMS) have been quantified on a total PCB basis as, when monitoring began, analytical technology was unable to separate, and hence identify, individual PCB congeners. Quantification was based on the comparison of the sum of all peak areas (excluding those compounds confirmed as organochlorine pesticides) in sample chromatograms with the total area of a technical Aroclor mixture (namely Aroclor 1254). The primary aim of the scheme has been to identify long-term temporal trends in contaminants in birds. To maintain consistency in reporting temporal trends and reduce the risk of introducing bias into the data set through changes in analytical methodologies, all subsequent PCB analysis has been reported in this way.

Summarising PCB concentrations on a total basis provides a necessary comparison with historical data from other studies. It is also a simple measure for identifying trends in overall PCB concentrations, as demonstrated in Chapter 3. However reporting PCB concentrations on a total PCB basis may not truly reflect changes in exposure as assimilation of some congeners may increase while assimilation of others may decrease, thus resulting in little change in total PCB concentration. Such scenarios could occur as individual PCB congeners vary in their environmental persistence and potential to bioaccumulate. Furthermore, given the wide range in toxicity between individual PCBs (Ahlborg et al., 1994), characterisation of the pattern of PCB exposure, as opposed to total PCB exposure, provides a more accurate evaluation of actual likely toxicity of exposure to PCBs.

PCB congener profiles have been reported for a limited number of sparrowhawks, kestrels (Naso et al., 2003) and grey herons (Jenssen et al., 2001; Boumphrey et al., 1993). These studies reported PCB congener patterns that were similar to those in other predatory bird species in that the profiles were dominated by PCB congeners 153, 138 and 180 (Kenntner et al., 2003b; Senthilkumar et al., 2002; Hoshi et al., 1998; Elliott et al., 1996b). Piscivorous species tend to have higher congener concentrations than other raptors (Naso et al., 2003; Senthilkumar et al., 2001; Hoshi et al., 1998), particularly for tri- and tetra-chlorinated congeners (Hoshi et al., 1998). However, differences between other groups of raptors may be less marked. Naso et al. (2003) reported similar congener profiles for a range of omnivorous and carnivorous bird species, and Manos et al. (2003) found no difference in the congener profiles of northern goshawks (*Accipiter gentiles*) and buzzards (*Buteo buteo*).

As with total and Aroclor-matched liver PCB residues (Kenntner et al., 2003a, 2003b; Newton et al., 1993), liver concentrations of PCB congeners can vary widely between individuals of the same species (Naso et al., 2003; Senthilkumar et al., 2001; Hoshi et al., 1998). In general, studies on PCB congener profiles in raptors have involved low (< 10) numbers of individuals for each species and so have not assessed in any depth how PCB profiles vary within species. However, Kenntner et al. (2003b) did report age and sex-related differences in the liver concentrations of six PCB congeners in goshawks. Adult birds had higher liver congener concentrations than juveniles and females generally had higher PCB concentrations than males.

The aim of the study described in this chapter was to quantify the PCB congener concentrations in sparrowhawks, kestrels and herons from the UK. This is the first time such national scale data have been generated for these species in the UK. Furthermore, the effect of body condition, age and sex on the magnitude of residues of individual

congeners was investigated as these factors had previously (Chapter 3) been shown to explain much of the intra-species variation in liver total PCB concentrations.

4.2 Method

A total of 404 sparrowhawk, 234 kestrel and 49 heron livers collected between 1992 and 1998 were analysed for sixteen individual PCB congeners, as detailed in Chapter 2. The congeners were those routinely analysed as part of the Predatory Bird Monitoring Scheme (PBMS) from 1992 onwards and were representative of the various PCB homologue groupings (di-chlorinated through to octa-chlorinated PCBs). They also included the seven PCB congeners routinely measured in fish by the International Council for the Exploration of the Sea (ICES) and the four most toxic non-*ortho*-substituted PCB congeners (Table 4.1). The limits of detection (Table 4.1) were determined as described in Chapter 2. PCB congener concentrations were $\log_{10}$ transformed prior to analysis to ensure data conformed to the underlying assumptions of the statistical models.

Table 4.1 PCB congener limits of detection for predatory bird livers analysed between 1992 and 1998. The four dioxin-like co-planar congeners and the ICES 7 PCB congeners are indicated by [a] and [b] respectively.

PCB congener	LOD/μg g^{-1}	PCB congener	LOD/μg g^{-1}
8	0.0035	126[a]	0.0020
18	0.0140	128	0.0005
28[b]	0.0045	138[b]	0.0006
31	0.0010	149	0.0025
52[b]	0.0029	153[b]	0.0015
77[a]	0.0020	169[a]	0.0002
101[b]	0.0010	170	0.0005
118[a,b]	0.0005	180[a]	0.0005

To simplify the assessment of nutritional state on PCB congener concentrations and to maximise the number of birds within different body condition classifications, birds assigned fat scores of 0 and 1 were combined into a single group which represented starved birds. Birds with fat scores between 2 and 5 were combined to form a second group that represented non-starved birds.

4.2.1 Initial summary of the data

For statistical purposes, non-detected values were assigned a value equal to the limit of detection for that congener. To provide an initial summary of the overall datasets and focus further statistical analysis on those congeners that were detected relatively frequently, the geometric mean concentrations and the frequency of detection for all congeners were calculated for each species. To determine whether the most frequently occurring congeners co-occurred, and whether the magnitude of co-occurring congener concentrations were associated, Pearson correlation coefficients were calculated for each pair of congeners that were detected in more than 20% of birds. Despite their low frequency of detection, the correlation analysis was expanded to include those non-*ortho*-substituted PCBs for which TEF values have been assigned (PCBs 77, 118, 126 and 169). These congeners were of particular interest because of their high toxicity, and were included so as to identify whether detectable levels of the four non-*ortho* congeners were correlated with concentrations of other PCB congeners. By conducting such an analysis, it would be possible to assess whether intra- and inter-species variation in concentrations of these more toxic PCBs were similar to, and so could be predicted from, other less potent but more prevalent congeners.

4.2.2 Causes of intra-species variation in liver PCB congener concentrations

Principal components analysis (PCA) was initially used to explore the main factors influencing variation in liver PCB congener concentrations. Subsequent analysis

involved examining the variation in concentrations of congeners that were detected in approximately more than 20% of all birds. A backwards stepwise General Linear Model (GLM) approach, as described in Chapter 3, was used. Body condition was a factor in the model, and age and sex were co-variables; the interaction terms between these variables were also included. All three terms had been identified in the PCA analysis as likely to affect liver congener concentrations. The model was run repeatedly and the least significant term removed after each run until the only terms remaining were those that were statistically significant. Factors/covariates that were not significant alone but were significant as interaction terms were retained in the model. A significance level of $p = 0.01$ was used for the sparrowhawk and kestrel data sets to minimise the likelihood of Type 1 statistical errors. The significance level was relaxed to $p = 0.05$ for the heron dataset as sample numbers were much lower.

4.2.3 Inter-species variation in liver PCB congener concentrations

Differences between species in the proportions of starved and non-starved birds with detectable concentrations of those congeners analysed in section 4.2.2 were compared by Chi-squared goodness-of-fit tests.

Sequential general linear model (GLM) analysis of each individual congener identified in section 4.2.2 was carried out to determine whether concentrations differed between sparrowhawks, kestrels and herons. The analysis was carried out separately on starved and non-starved birds. Congener concentration was the response variable, age was a covariate and species was the predictor variable.

4.3 Results

4.3.1 PCB congener concentrations and occurrence in predatory bird livers

Overall, PCBs 153, 138 and 180 were the most frequently detected and occurred in some 86% of the sparrowhawks and 94% of the herons analysed. These congeners also occurred in the highest concentrations and accounted for 75% (sparrowhawks) and 76% (herons) of the summed congener concentrations (Figures 4.1 and 4.2 respectively). Mean concentrations of PCBs 101 and 169 contributed least to the summed congener concentrations but these congeners were detected in greater proportions of birds (> 26% sparrowhawks and > 14% herons) than other PCBs that had higher mean concentrations.

The congener pattern of kestrels differed from those of sparrowhawks and herons in that the mean concentrations of two dichloro-PCB congeners (8 and 18) were of similar magnitude to those of the more persistent higher chlorinated congeners, despite occurring in less than 2% of kestrels analysed (Figure 4.3). It is likely the mean concentrations of these two congeners were an artefact, and arose because the values assigned to non-detected concentrations were relatively high, reflecting their relatively high limits of detection. Thus the high mean value reflected the large proportion of non-detected concentrations for these congeners rather than true liver concentrations. This anomaly was also evident in the sparrowhawk and heron datasets as these congeners were amongst the least-frequently detected congeners yet their mean concentrations appeared to occur at mid-ranked positions in the overall congener profiles (Figures 4.1 and 4.2). When these two congeners were discounted from the kestrel profile, the pattern was similar to that of the sparrowhawks and herons with PCBs 153, 138 and 180 occurring at the highest concentrations and most frequently. These three congeners accounted for 40% of the mean sum congener concentration in kestrels.

Of the dioxin-like PCBs analysed, 118 was the most prevalent in all three species whilst congeners 77, 126 and 169 were amongst those congeners that occurred at lowest concentrations and in relatively few birds (Figures 4.1 to 4.3).

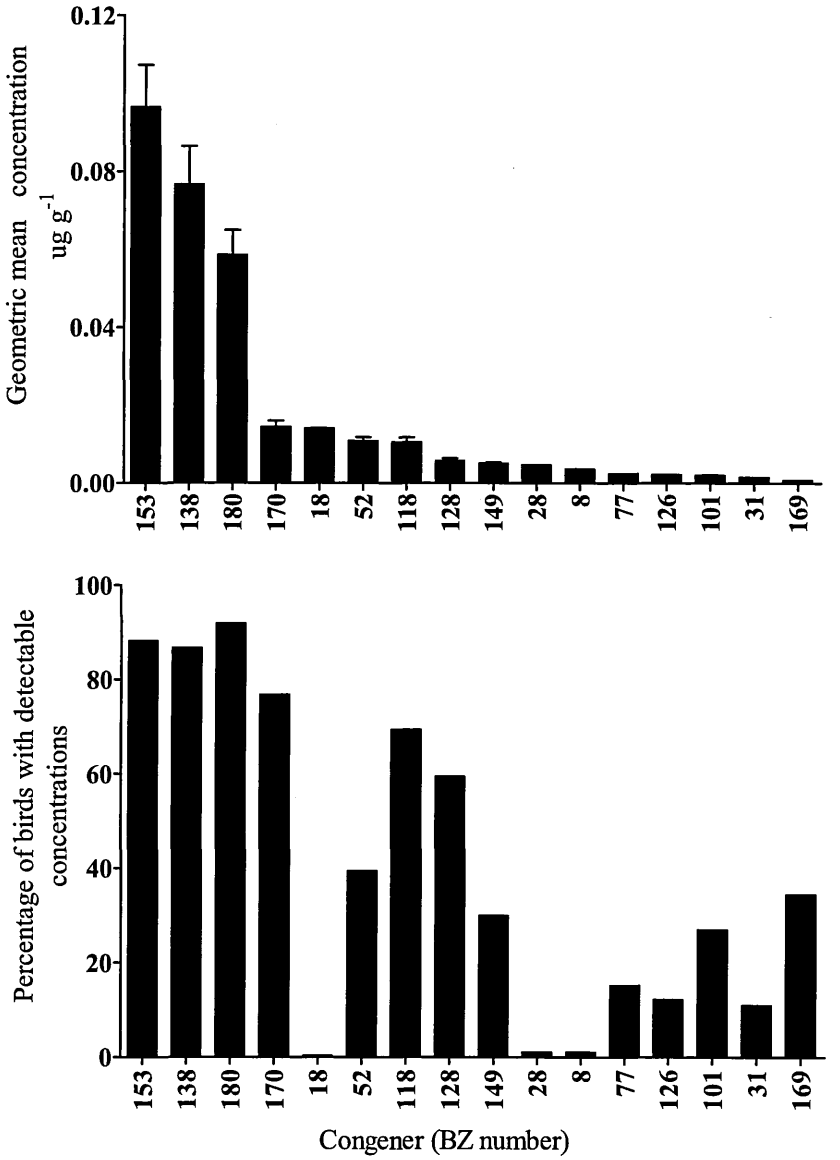

Figure 4.1 Geometric mean (+GSE) PCB congener concentrations and frequency of occurrence in sparrowhawk livers.

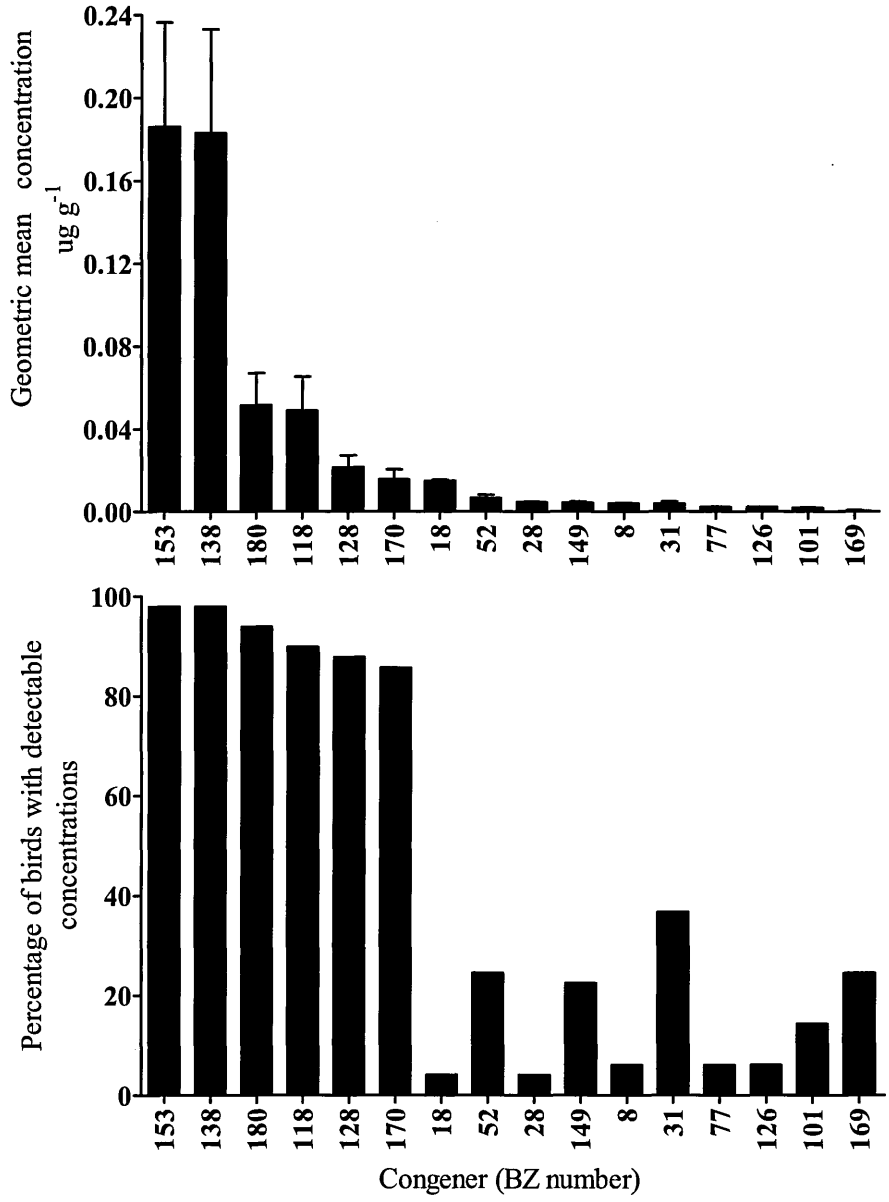

Figure 4.2 Geometric mean (+GSE) PCB congener concentrations and frequency of occurrence in heron livers.

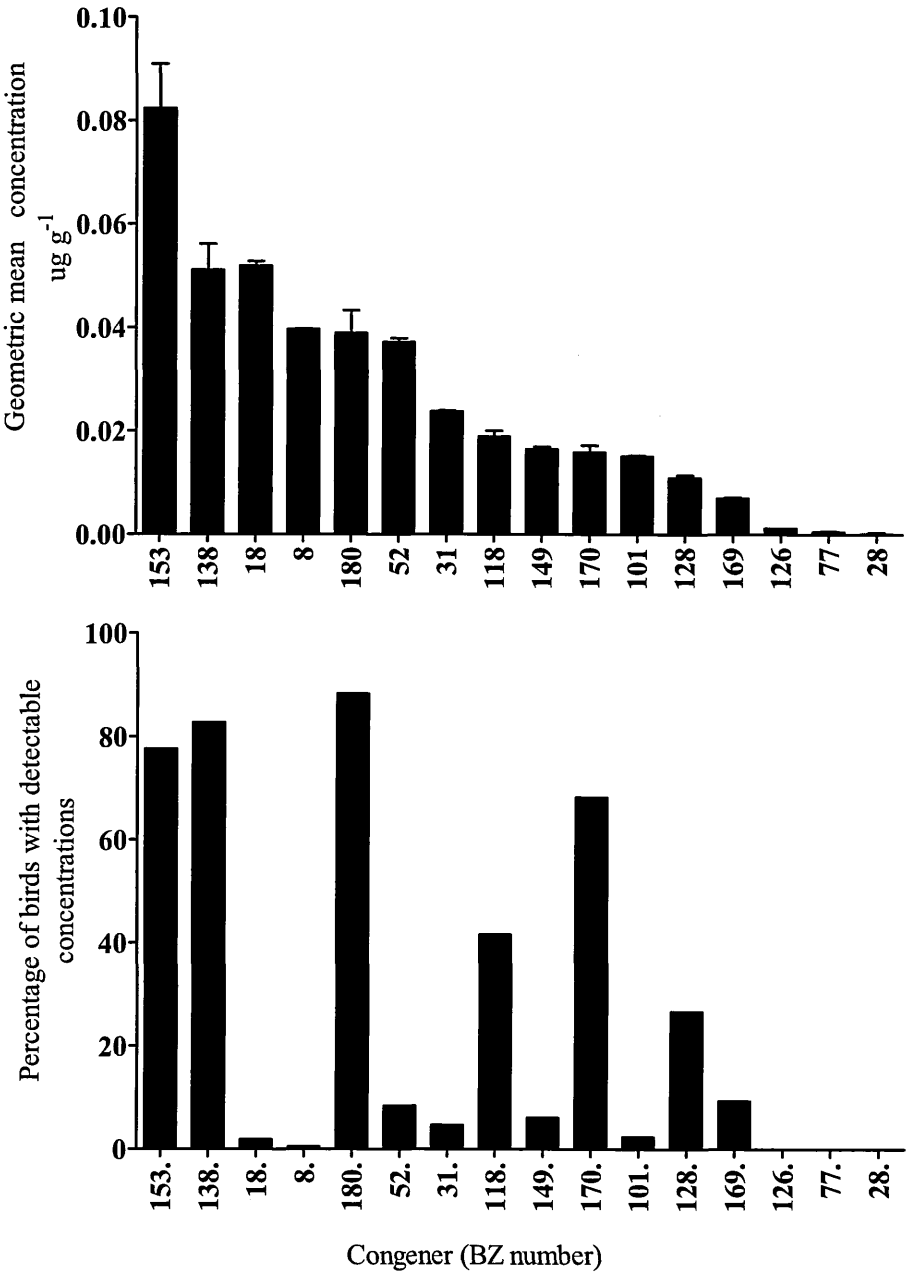

Figure 4.3 Geometric mean (+GSE) PCB congener concentrations and frequency of occurrence in kestrel livers.

In general, the liver concentrations of the most prevalent congeners (153, 138, 180, 170, 118 and 128) were all highly positively correlated with each other in sparrowhawks, herons and kestrels (Tables 4.2 to 4.4). Scatter plots depicting the correlations are provided in Appendix 1. In sparrowhawks and herons, the liver concentrations of PCBs 52, 149 and 169 were also weakly positively correlated with all congeners that were detected in more than 20% of birds.

Table 4.2 Pearson correlation coefficients between liver PCB congener log_{10} concentrations in sparrowhawks (coefficients highlighted in bold were statistically significant ($p < 0.01$)).

PCB#	153	138	180	170	118	128	52	169	149	101	77
138	**0.732**										
180	**0.725**	**0.778**									
170	**0.760**	**0.814**	**0.812**								
118	**0.659**	**0.785**	**0.733**	**0.802**							
128	**0.698**	**0.764**	**0.674**	**0.831**	**0.826**						
52	**0.419**	**0.529**	**0.367**	**0.476**	**0.511**	**0.554**					
169	**0.242**	**0.438**	**0.406**	**0.417**	**0.472**	**0.438**	**0.301**				
149	**0.400**	**0.460**	**0.414**	**0.416**	**0.530**	**0.518**	**0.509**	**0.461**			
101	**0.414**	**0.416**	**0.366**	**0.430**	**0.464**	**0.490**	**0.349**	**0.313**	**0.527**		

Table 4.3 Pearson correlation coefficients between liver PCB congener log_{10} concentrations in herons (coefficients highlighted in bold were statistically significant ($p < 0.01$)).

PCB#	153	138	180	118	128	170	169	52	149	77
138	**0.944**									
180	**0.866**	**0.785**								
118	**0.900**	**0.859**	**0.803**							
128	**0.810**	**0.869**	**0.750**	**0.767**						
170	**0.908**	**0.839**	**0.826**	**0.900**	**0.786**					
169	**0.414**	0.316	**0.371**	0.347	0.246	**0.407**				
52	**0.542**	**0.568**	0.309	**0.486**	**0.457**	**0.446**	**0.472**			
149	**0.406**	**0.477**	ns	**0.415**	**0.439**	**0.367**	0.216	**0.425**		

Table 4.4 Pearson correlation coefficients between liver PCB congener log_{10} concentrations in kestrels (coefficients highlighted in bold were statistically significant ($p < 0.01$)).

PCB#	180	138	153	170	118	128	52	169	149	101
138	**0.790**									
153	**0.892**	**0.838**								
170	**0.910**	**0.840**	**0.897**							
118	**0.628**	**0.748**	**0.694**	**0.736**						
128	**0.543**	**0.678**	**0.620**	**0.669**	**0.807**					

4.3.2 Intra-species variation in liver PCB congener concentrations

PCA analysis of the sparrowhawk data showed that the first two principal components accounted for only 40% of the variation in liver PCB concentrations (eigenvalues were 6.33 and 1.64 respectively, Figure 4.4). Results of the PCA analysis of kestrel and heron data were similar, with only 35% and 42% respectively of the variance in liver congener concentrations explained by the first two principal components (Figures 4.6 and 4.8). Fat score appeared to explain the majority of the variation along the first principal component for all species (Figures 4.5, 4.7 and 4.9), but there were no other obvious patterns in the data. Therefore a general linear model approach (GLM) as used in Chapter 3 was employed to further investigate sources of intra-species variation in liver congener concentrations.

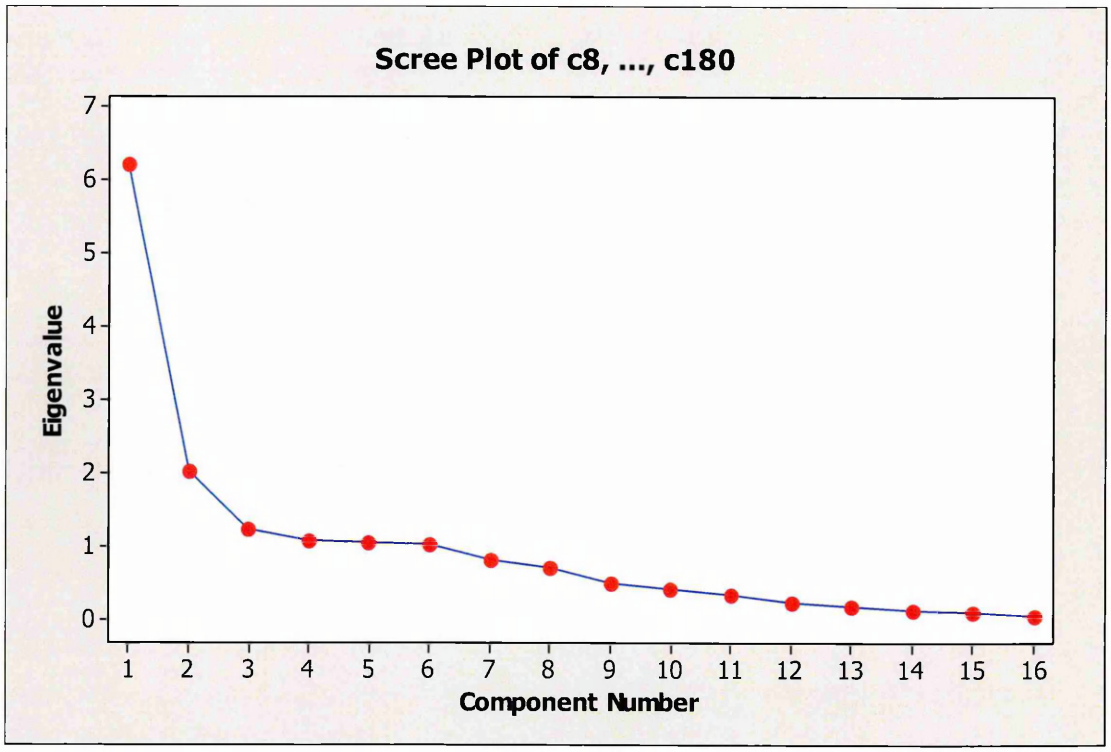

Figure 4.4 Scree plot for principal component analysis of liver PCB congener concentrations in sparrowhawks.

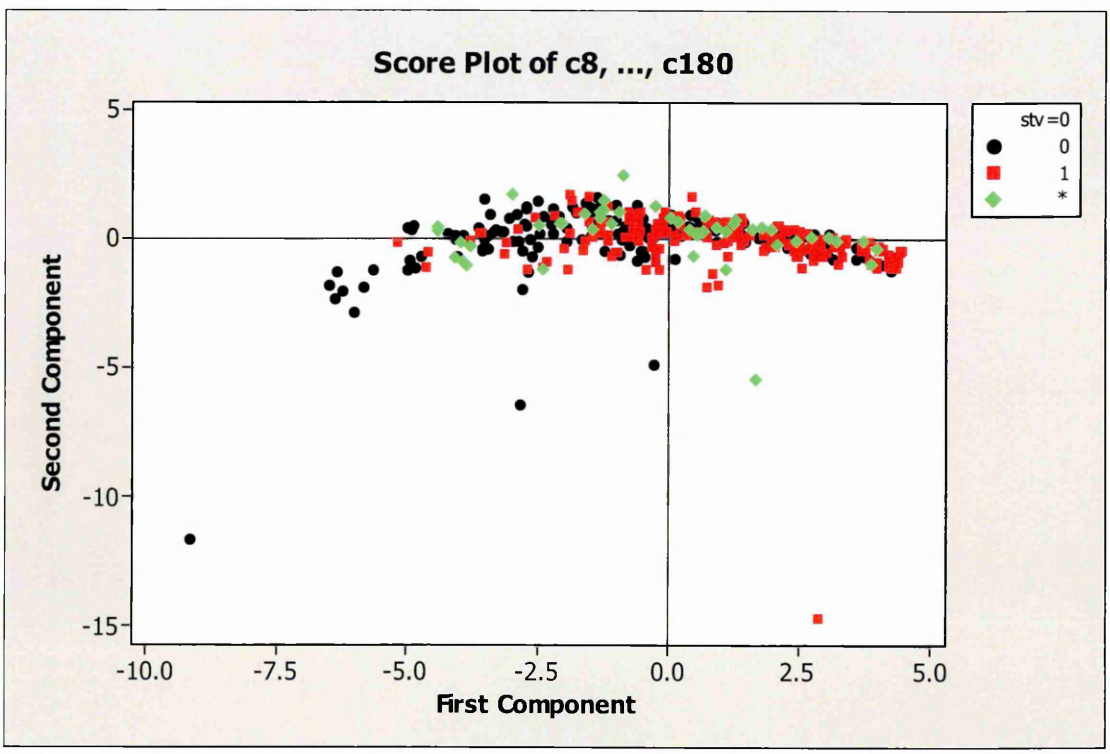

Figure 4.5 PCB congener score plot results of principal component analysis of liver PCB concentrations in sparrowhawks (black circles = starved birds, red squares = non-starved birds, green diamonds = undetermined body condition).

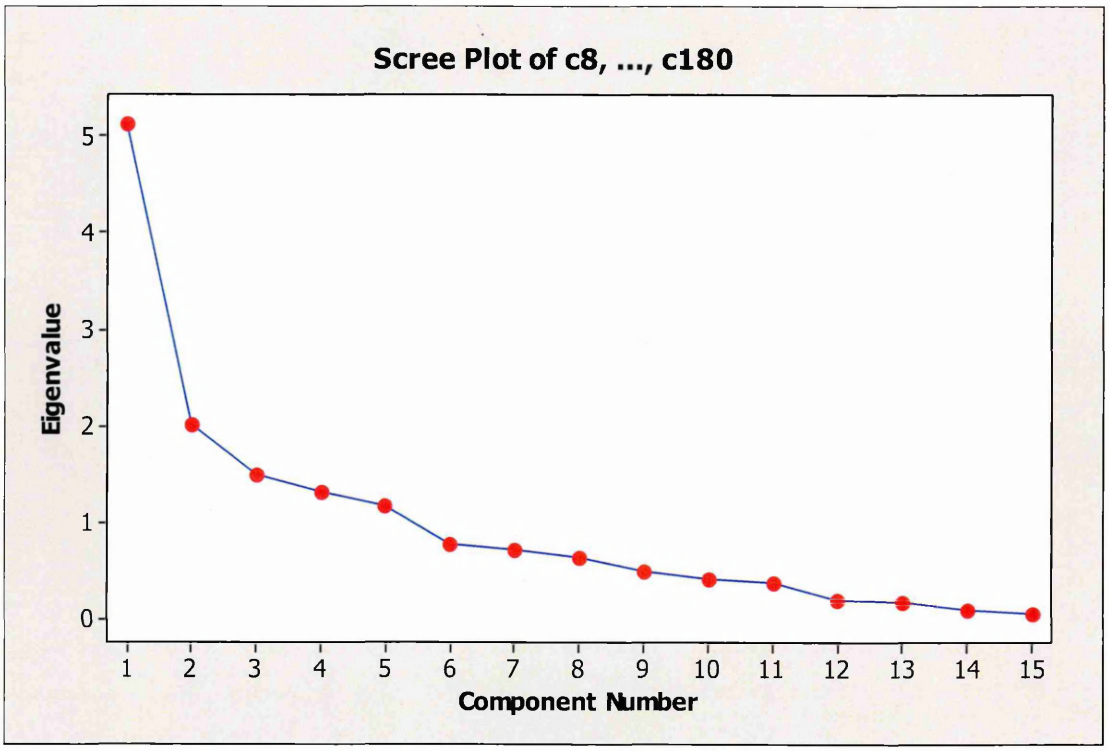

Figure 4.6 Scree plot for principal component analysis of liver PCB congener concentrations in kestrels.

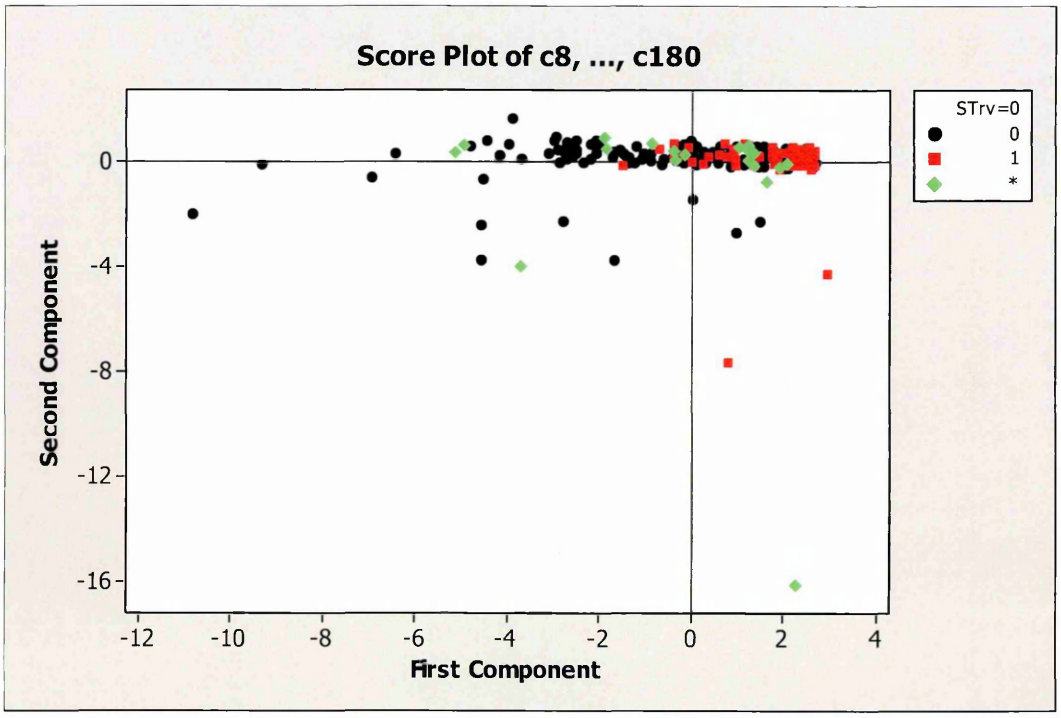

Figure 4.7 PCB congener score plot results of principal component analysis of liver PCB concentrations in kestrels (black circles = starved birds, red squares = non-starved birds, green diamonds = undetermined body condition).

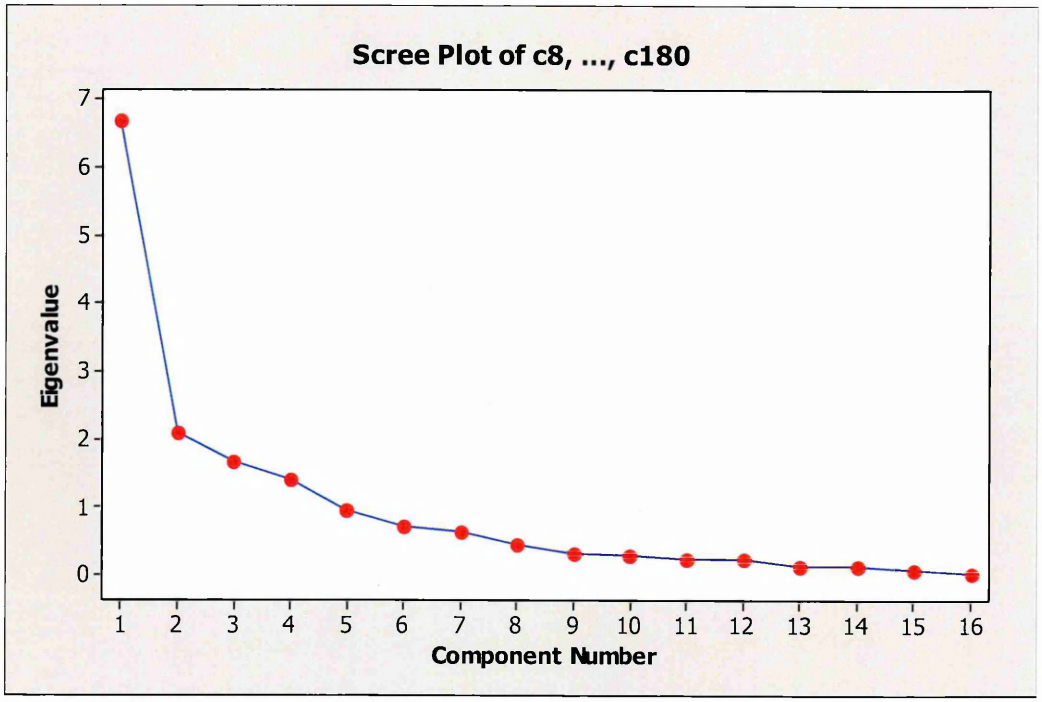

Figure 4.8 Scree plot for principal component analysis of liver PCB congener concentrations in herons.

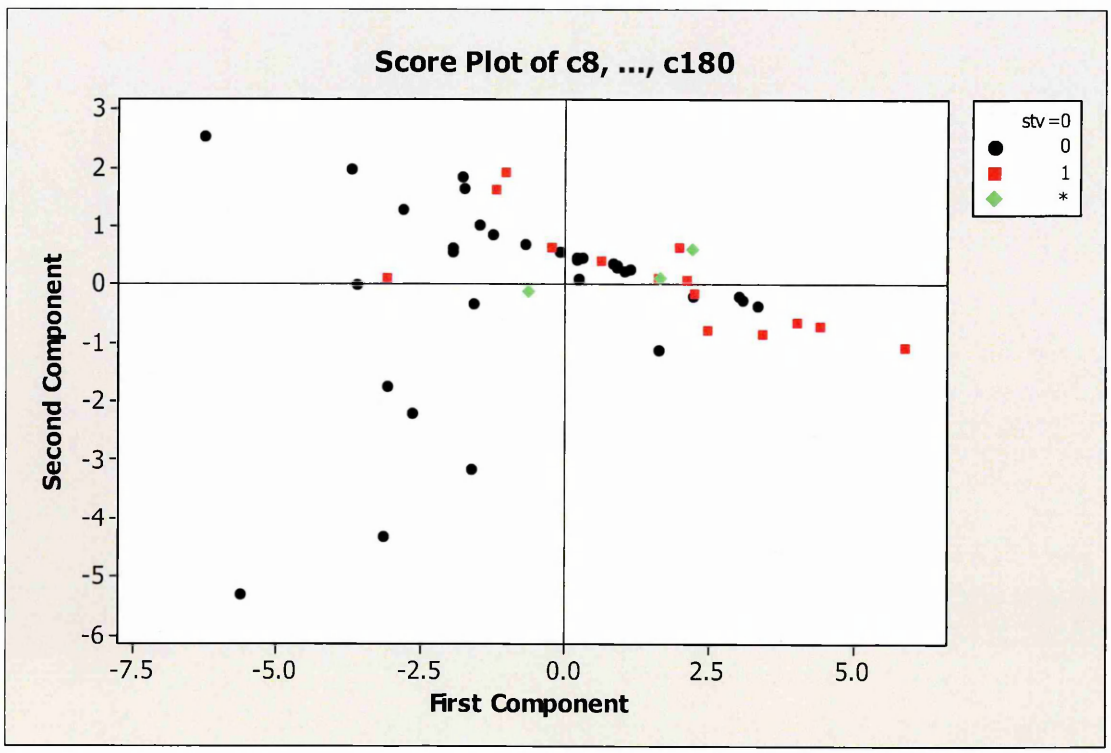

Figure 4.9 PCB congener score plot results of principal component analysis of liver PCB concentrations in herons (black circles = starved birds, red squares = non-starved birds, green diamonds = undetermined body condition).

In sparrowhawks, body condition significantly influenced liver concentrations of all PCB congeners either directly or as part of an interaction term (Table 4.5). As with total PCB concentrations (Chapter 3), liver concentrations of individual congeners were significantly higher in starved than non-starved birds (Figures 4.10 and 4.11). Age too was a significant factor that affected liver concentrations of all the congeners that were analysed (Table 4.5). Adults had higher congener concentrations than first-year birds (Figures 4.10 and 4.11). Sex also explained some of the variation in liver concentrations but only for congeners 118, 170 and 180. Liver concentrations were higher in males than females and the differences for congeners 170 and 180 were more marked in non-starved than starved birds, as indicated by the significant sex*body condition interaction

Table 4.5 The results of general linear model analysis of intra-species variation in liver PCB congener concentrations in sparrowhawks.

PCB #	Body condition	Age	Sex	Body condition*age	Body condition*sex
52	$F_{(1,344)} = 1.66$ $p > 0.01$	$F_{(1,344)} = 9.93$ $p < 0.01$	$F_{(1,344)} = 0.02$ $p > 0.01$		$F_{(1,344)} = 12.09$ $p < 0.01$
101	$F_{(1,344)} = 10.30$ $p < 0.01$	$F_{(1,344)} = 21.54$ $p < 0.01$			
118	$F_{(1,344)} = 40.21$ $p < 0.01$	$F_{(1,344)} = 34.44$ $p < 0.01$	$F_{(1,344)} = 7.77$ $p < 0.01$		
128	$F_{(1,344)} = 47.94$ $p < 0.01$	$F_{(1,344)} = 51.69$ $p < 0.01$			
138	$F_{(1,344)} = 33.34$ $p < 0.01$	$F_{(1,344)} = 45.90$ $p < 0.01$			
149	$F_{(1,344)} = 40.78$ $p < 0.01$	$F_{(1,344)} = 13.84$ $p < 0.01$			
153	$F_{(1,344)} = 59.87$ $p < 0.01$	$F_{(1,344)} = 39.73$ $p < 0.01$			
169	$F_{(1,344)} = 4.21$ $p > 0.01$	$F_{(1,344)} = 23.39$ $p < 0.01$	$F_{(1,344)} = 7.52$ $p < 0.01$	$F_{(1,344)} = 8.61$ $p < 0.01$	
170	$F_{(1,344)} = 7.89$ $p < 0.01$	$F_{(1,344)} = 55.76$ $p < 0.01$	$F_{(1,344)} = 3.64$ $p > 0.01$		$F_{(1,344)} = 6.54$ $p < 0.01$
180	$F_{(1,344)} = 10.24$ $p < 0.01$	$F_{(1,344)} = 62.75$ $p < 0.01$	$F_{(1,344)} = 5.11$ $p > 0.01$		$F_{(1,344)} = 7.19$ $p < 0.01$

term for these congeners (Table 4.5, Figures 4.10 and 4.11). Liver concentrations of congeners 153 and 138 also appeared to be higher in non-starved males than non-starved females, but neither sex nor the sex*body condition interaction term were statistically significant for either congener.

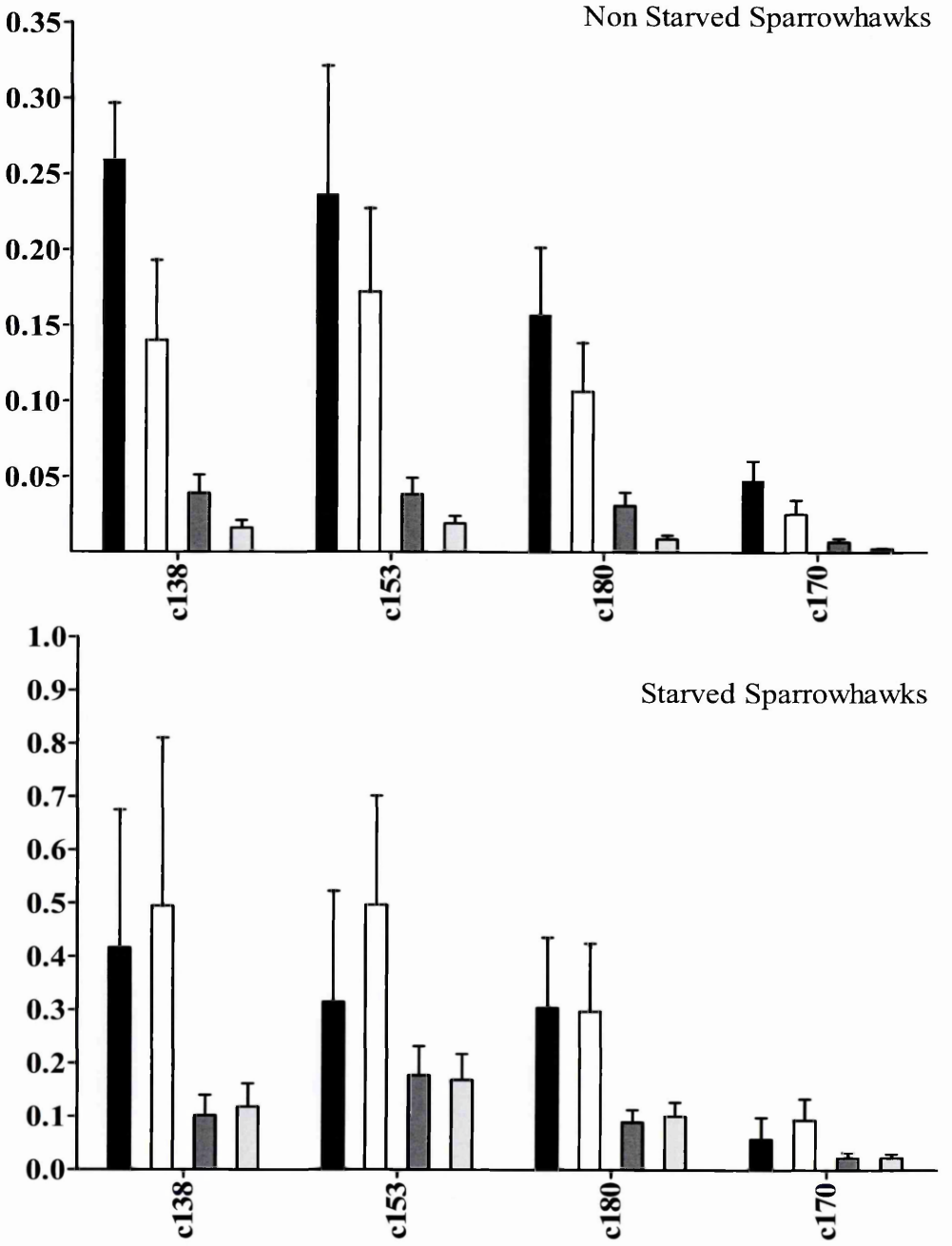

Figure 4.10 Geometric mean (+GSE) liver concentrations (μg g^{-1}) of PCB congeners 138, 153, 180 and 170 in non-starved and starved sparrowhawks. Birds are separated into adult male (black bars) and females (white bars), first-year male (dark grey bars) and females (pale grey bars).

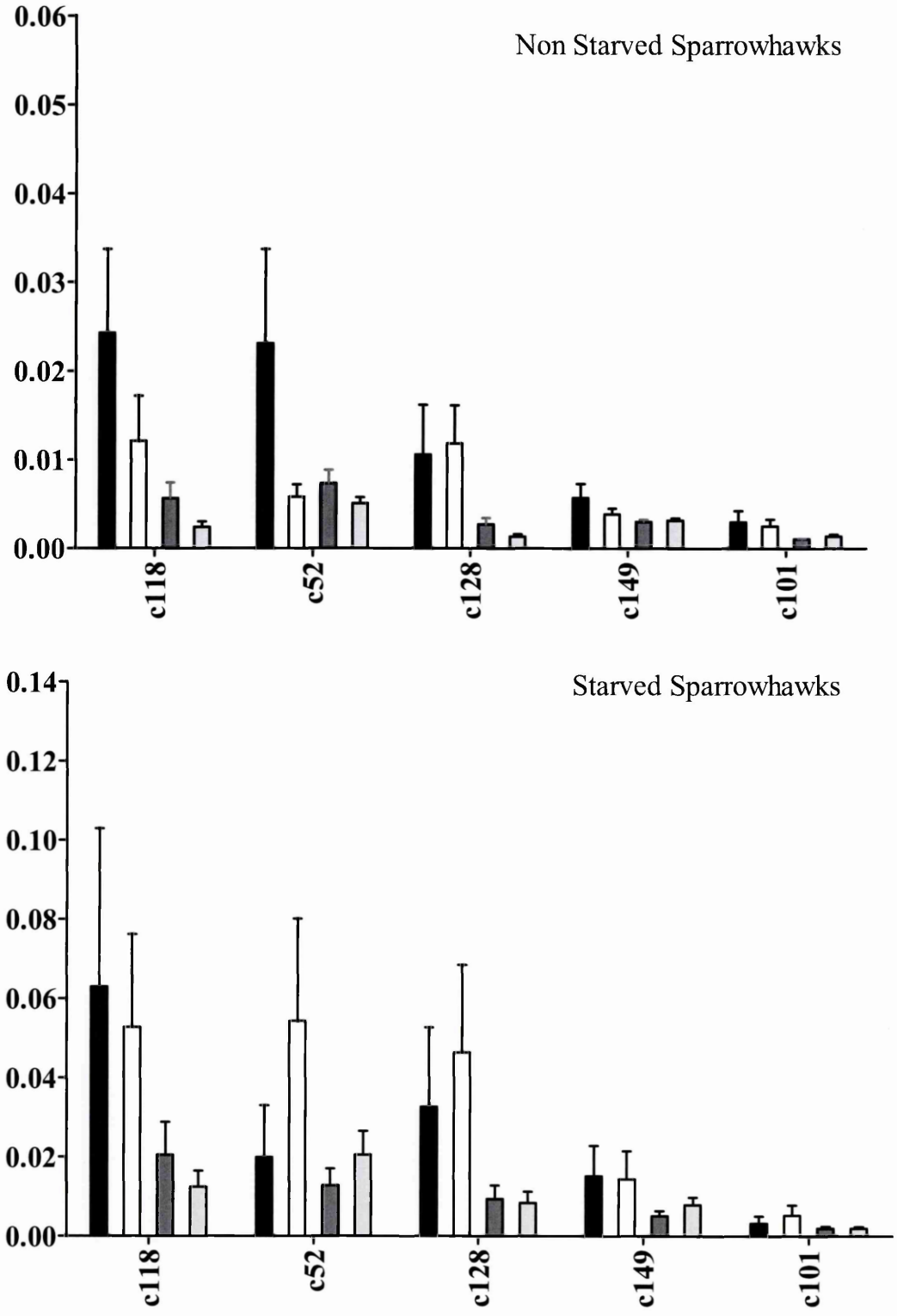

Figure 4.11 Geometric mean (+GSE) liver concentrations (μg g^{-1}) of PCB congeners 118, 52, 128, 149, and 101 in non-starved and starved sparrowhawks. Birds are separated into adult male (black bars) and females (white bars), first-year male (dark grey bars) and females (pale grey bars)

Overall, body condition, age and sex all significantly influenced the magnitude of liver PCB congener concentrations in sparrowhawks. Nevertheless, the congener profile remained relatively similar between individuals, in that congeners 153, 138 and 180 occurred in the highest concentrations in all birds. A second group of congeners, PCBs 170, 118, 128, 52 and 149, occurred at concentrations approximately an order of magnitude lower than PCBs 153, 138 and 180. The remaining congeners (PCBs 101, 169, 77, 31 and 126) were detected at trace levels, close to their limits of detection.

Kestrel liver congener concentrations varied significantly with both body condition and age (Table 4.6) for congeners that were detected in more than 20% of birds. Starved kestrels generally had higher liver concentrations than non-starved birds and adults higher congener concentrations than first-year birds (Figure 4.12). However, the effect of age depended on body condition in that the difference between first-years and adults was greater in starved than non-starved birds. Hence, although age alone was a highly significant factor in explaining congener concentrations, the GLM analysis highlighted a significant interaction between age and body condition.

Table 4.6 The results of general linear model analysis of intra-species variation in liver PCB congener concentrations in kestrels.

PCB #	Body condition	Age	Sex	Body condition*age	Body condition*sex
118	$F_{(1,191)} = 9.65$ $p < 0.01$	$F_{(1,191)} = 13.35$ $p < 0.01$		$F_{(1,191)} = 15.72$ $p < 0.01$	
128	$F_{(1,191)} = 23.30$ $p < 0.01$	$F_{(1,191)} = 7.30$ $p < 0.01$			
138	$F_{(1,191)} = 7.48$ $p < 0.01$	$F_{(1,191)} = 8.27$ $p < 0.01$		$F_{(1,191)} = 9.15$ $p < 0.01$	
153	$F_{(1,191)} = 5.01$ $p > 0.01$	$F_{(1,191)} = 9.86$ $p < 0.01$	$F_{(1,191)} = 1.53$ $p > 0.05$	$F_{(1,191)} = 15.06$ $p < 0.01$	$F_{(1,191)} = 8.97$ $p < 0.01$
170	$F_{(1,191)} = 22.97$ $p < 0.01$	$F_{(1,191)} = 16.33$ $p < 0.01$		$F_{(1,191)} = 12.64$ $p < 0.01$	
180	$F_{(1,191)} = 5.70$ $p > 0.01$	$F_{(1,191)} = 14.38$ $p < 0.01$	$F_{(1,191)} = 2.82$ $p > 0.01$	$F_{(1,191)} = 11.99$ $p < 0.01$	$F_{(1,191)} = 13.09$ $p < 0.01$

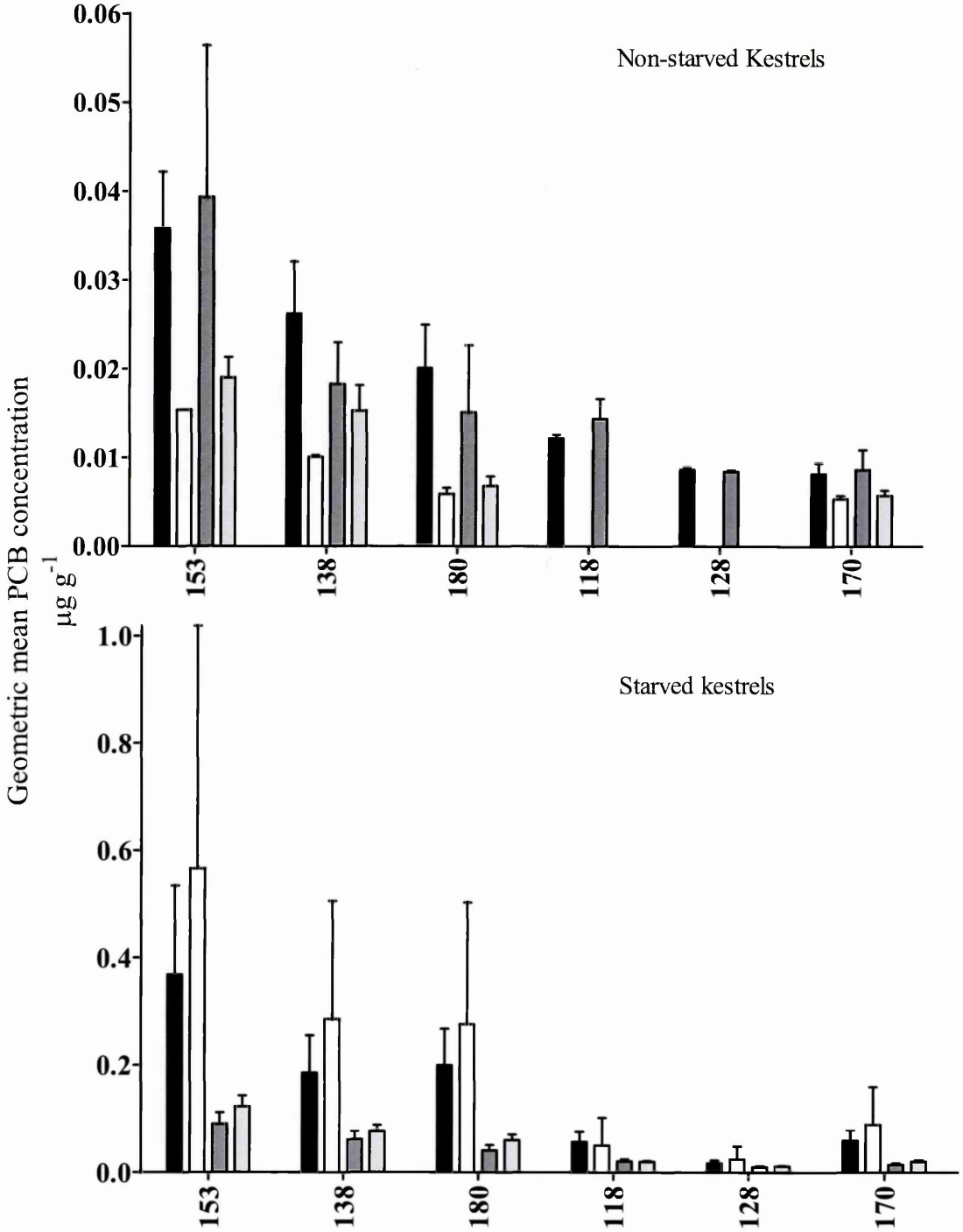

Figure 4.12 Geometric mean (+GSE) liver concentrations (µg g^{-1}) of PCB congeners in non-starved and starved kestrels. Birds are separated into adult male (black bars) and females (white bars), first-year male (dark grey bars) and females (pale grey bars).

Sex significantly influenced the liver concentrations of four congeners (PCBs 153, 138, 170 and 180) but not as a factor on its own. As with age, there was a significant interaction between sex and body condition. In non-starved kestrels, liver

concentrations of these four congeners were higher in males than in females, whilst the difference between males and females was much less marked in starved birds (Figure 4.12). Overall the pattern of PCB congeners was similar between different ages and sexes within each body condition class. Congener profiles differed between non-starved and starved kestrels by the absence of PCBs 118, 128, 169, 149 and 52 from non-starved birds.

In herons, as with kestrels and sparrowhawks, body condition significantly explained much of the variation in most congener concentrations either directly or through interaction terms (Table 4.7), but this was not true of PCB 149. Starved herons generally had higher liver concentrations than non-starved herons (Figures 4.13 and 4.14). Liver concentrations of PCBs 153, 169 and 180 were also significantly influenced

Table 4.7 The results of General Linear Model analysis of intra-species variation in liver PCB congener concentrations in herons.

PCB #	Body condition	Age	Sex	Body condition*age	Body condition*sex
31	$F_{(1,45)} = 4.21$ $p < 0.05$				
52	$F_{(1,45)} = 1.51$ $p < 0.05$				
118	$F_{(1,45)} = 6.83$ $p < 0.05$				
128	$F_{(1,45)} = 12.01$ $p < 0.05$				
138	$F_{(1,45)} = 11.00$ $p < 0.05$				
149	$F_{(1,45)} = 2.12$ $p > 0.05$				
153	$F_{(1,45)} = 4.41$ $p < 0.05$	$F_{(1,45)} = 0.647$ $p > 0.05$		$F_{(1,45)} = 4.38$ $p < 0.05$	
169	$F_{(1,45)} = 2.72$ $p > 0.05$	$F_{(1,45)} = 0.88$ $p > 0.05$	$F_{(1,45)} = 4.65$ $p < 0.05$	$F_{(1,45)} = 4.36$ $p < 0.05$	$F_{(1,45)} = 5.50$ $p < 0.05$
170	$F_{(1,45)} = 10.09$ $p < 0.05$				
180	$F_{(1,45)} = 1.52$ $p > 0.05$	$F_{(1,45)} = 1.20$ $p > 0.05$		$F_{(1,45)} = 6.25$ $p < 0.05$	

by an interaction between body condition and age in that non-starved adults had higher concentrations of PCBs than non-starved first-year herons whilst the opposite was true for starved herons. Sex was only a significant factor explaining variation in liver concentrations for congener 169. Residues were higher in females than in males and this

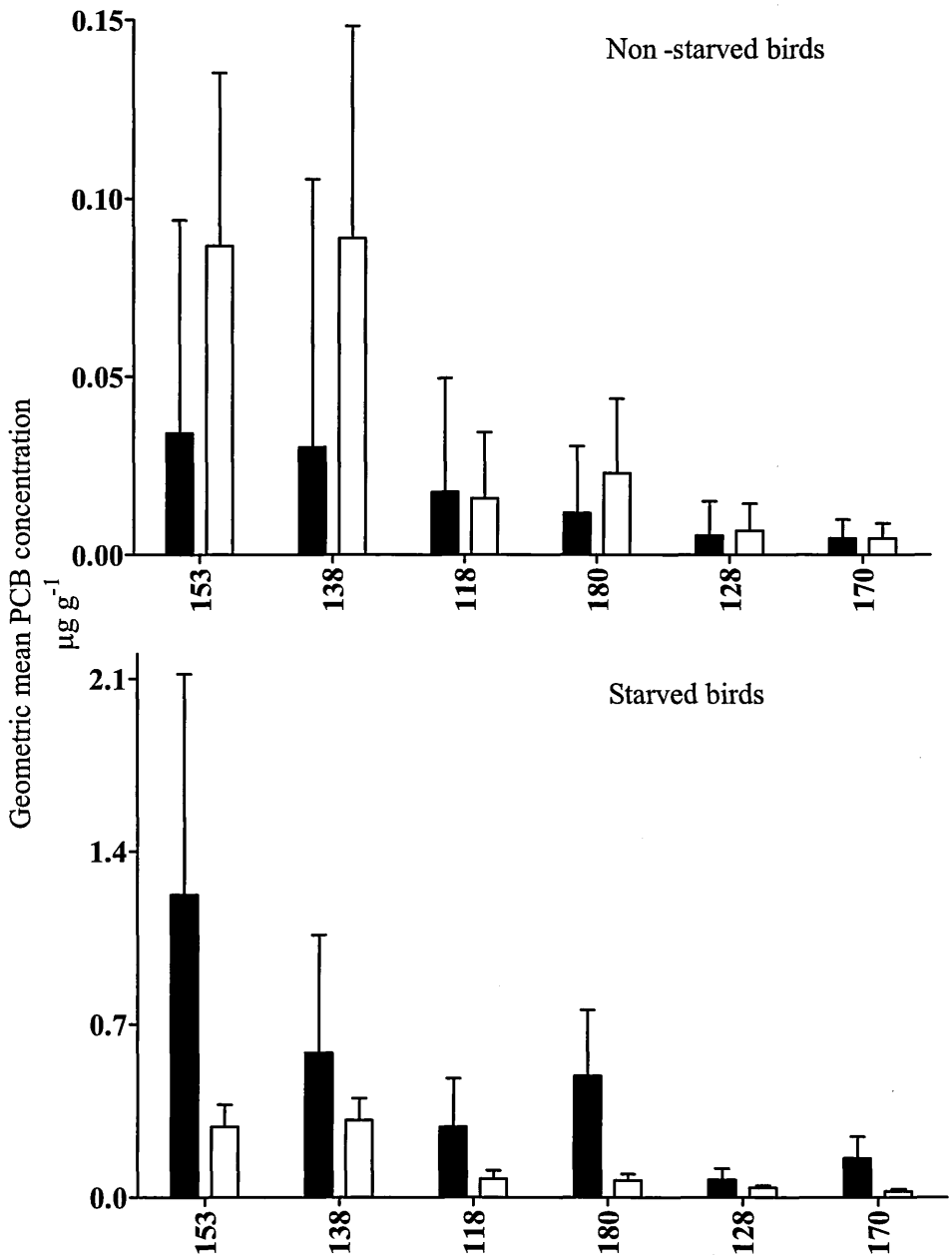

Figure 4.13 Geometric mean (+GSE) liver concentrations (µg g^{-1}) of PCB congeners 153, 138, 118, 180, 128 and 170 in non-starved and starved herons. Birds are separated into adults (black bars) and first-year birds (white bars).

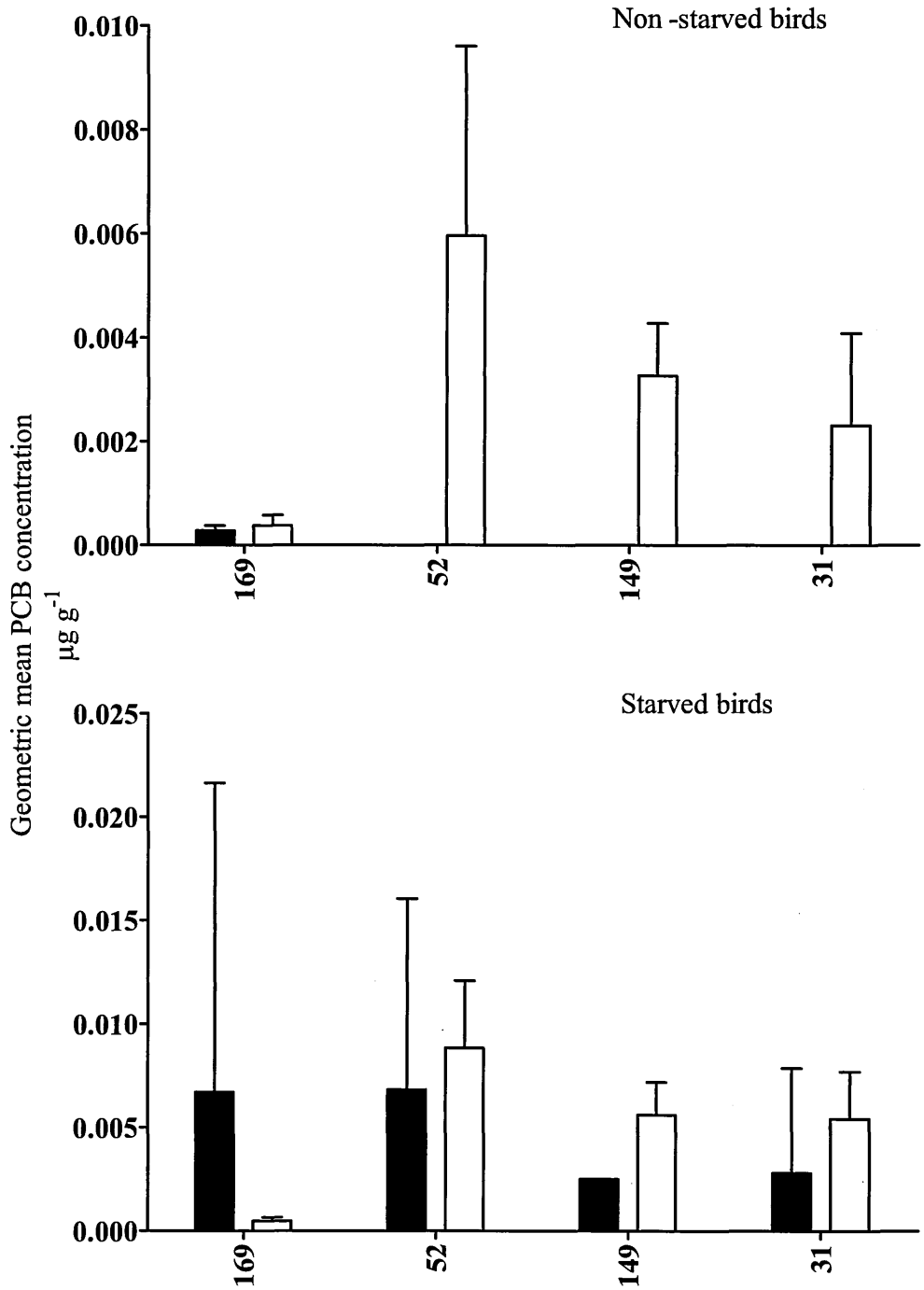

Figure 4.14 Geometric mean (+GSE) liver concentrations (µg g^{-1}) of PCB congeners 169, 52, 149, and 31 in non-starved and starved herons. Birds are separated into adults (black bars) and first-year birds (white bars).

difference was greater in starved than non-starved birds, as indicated by the significant interaction term. As with the sparrowhawk data, body condition affected the magnitude of PCB congener concentrations but not the overall pattern of occurrence.

The degree to which PCB congeners were influenced by body condition varied between congeners. Overall for sparrowhawks, kestrels and herons, the increase in liver concentrations of PCBs 118, 128, 138, 153, 170 and 180 in starved than non-starved birds was proportionally greater than for other congeners (Figure 4.15).

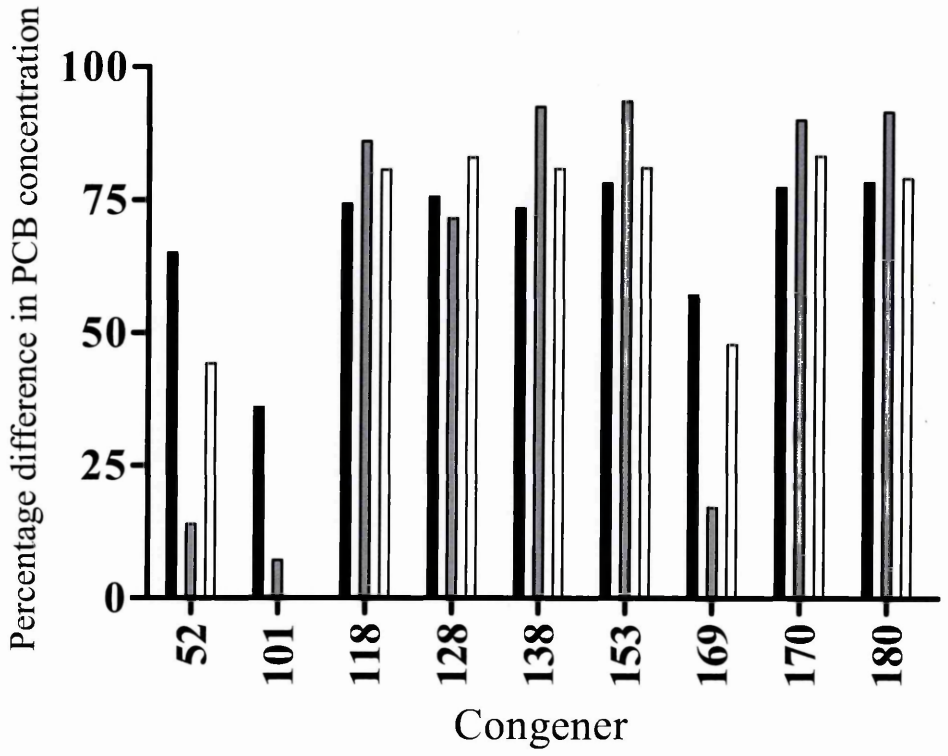

Figure 4.15 The percentage difference in congener concentration between non-starved and starved sparrowhawks (black bars), kestrels (grey bars) and herons (white bars).

4.3.3 Inter-species variation in liver PCB congener concentrations

Analysis of the proportions of birds with detectable concentrations of each congener indicated that there were significant species differences in the frequency of detection of several congeners. In non-starved birds, the proportions of birds with detectable concentrations of congeners 118, 128 and 138 varied between sparrowhawks, kestrels and herons ($\chi^2 > 9.4$, $p < 0.01$ in all; Figure 4.16). In starved birds, there were also species difference in the proportion of birds with detectable concentrations of congeners 31, 52, 101 and 149 as well as 118 and 128 ($\chi^2 > 15.7$, $p < 0.001$ in all) but no species difference for congener 138 (Figure 4.17).

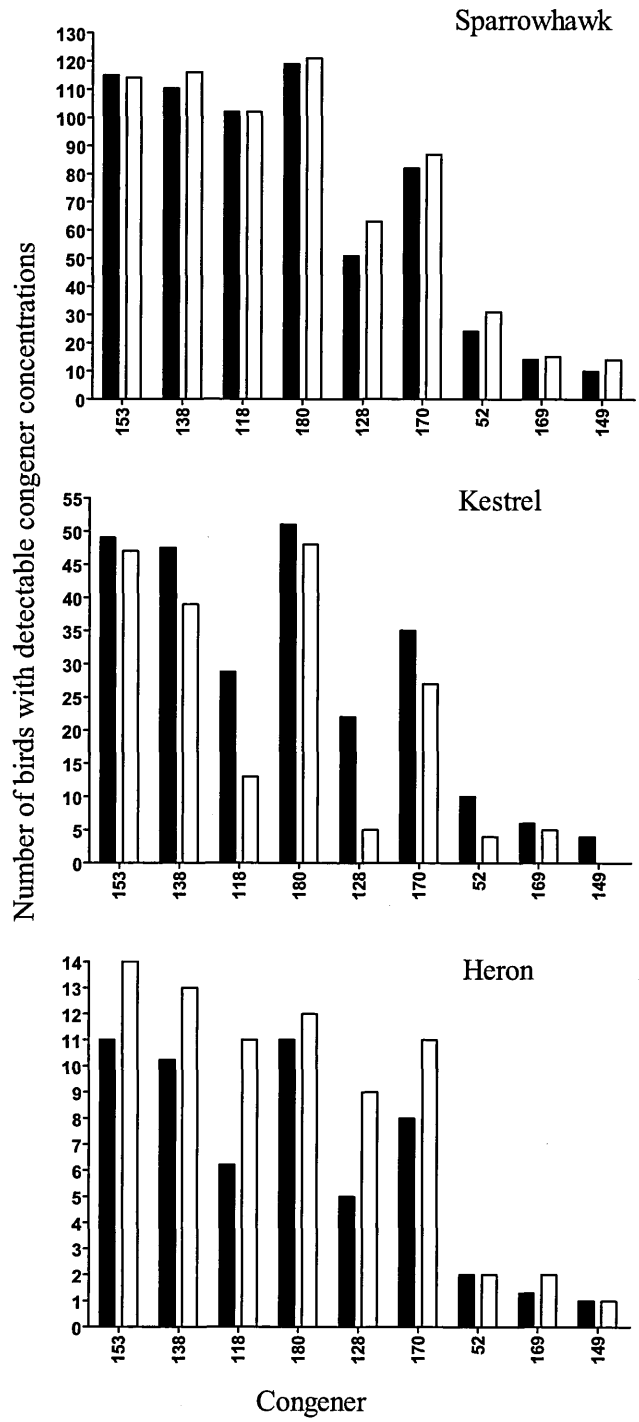

Figure 4.16 Frequency of detection of PCB congeners in non-starved predatory birds. χ^2 expected counts are represented by black bars, actual number of birds with detectable congener concentrations are represented by white bars.

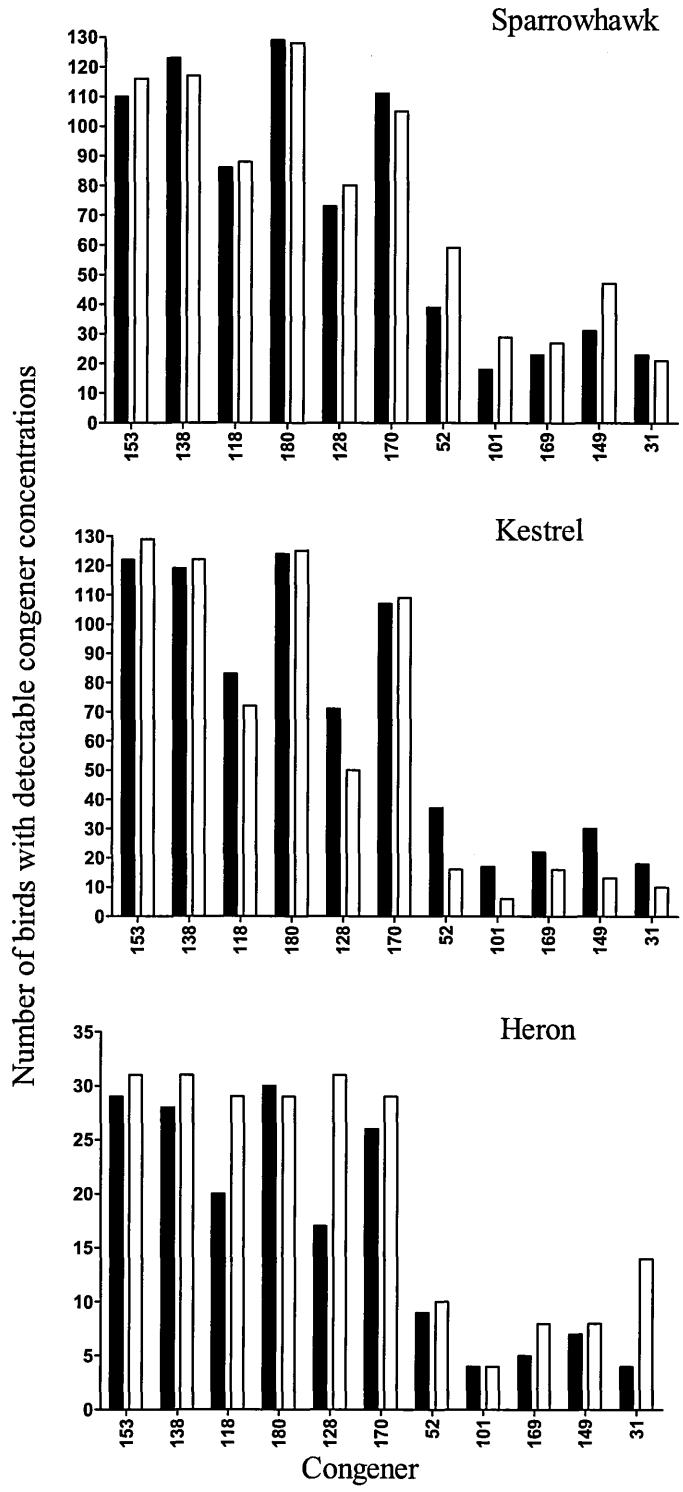

Figure 4.17 Frequency of detection of PCB congeners in starved predatory birds. χ^2 expected counts are represented by black bars, actual number of birds with detectable congener concentrations are represented by white bars.

In non-starved birds, liver PCB congener concentrations varied significantly between sparrowhawks, kestrels and herons (Table 4.8, Figures 4.18 and 4.19). Overall, kestrels had significantly lower liver PCB concentrations of congeners 118, 128, 138 and 153 than either sparrowhawks or herons (Bonferroni simultaneous tests $T > 3.99$, $p < 0.001$ in all). Liver concentrations of PCBs 52, 169, 170 and 180 were significantly lower in kestrels than sparrowhawks ($T > 3.14$, $p < 0.005$ in all) but did not differ between kestrels and herons ($T < 2.33$, $p > 0.05$ in all). Age had previously been identified as a significant factor explaining within species variation in liver concentrations. GLM analysis of between species variation highlighted that there was also a significant interaction between age and species for congeners 128, 138, 153, 170 and 180 liver concentrations (Table 4.8). This indicated that differences in liver concentrations of these congeners between adult and first-year birds were species specific. In general, the liver congener concentrations were ten-fold higher in adult than in first-year sparrowhawks whereas concentrations in adult and first-year birds were of a similar magnitude for both kestrels and herons (Figures 4.18 and 4.19).

Table 4.8 The results of general linear model analysis of inter-species variation in liver PCB congener concentrations in non-starved predatory birds.

PCB #	Species	Age	Age*species
52	$F_{(1,269)} = 7.62$ $p < 0.01$	$F_{(1,269)} = 1.61$ $p > 0.01$	$F_{(1,269)} = 1.87$ $p > 0.01$
118	$F_{(1,269)} = 34.98$ $p < 0.01$	$F_{(1,269)} = 9.18$ $p < 0.01$	$F_{(1,269)} = 3.33$ $p > 0.01$
128	$F_{(1,269)} = 34.92$ $p < 0.01$	$F_{(1,269)} = 20.24$ $p < 0.01$	$F_{(1,269)} = 6.87$ $p < 0.01$
138	$F_{(1,269)} = 34.37$ $p < 0.01$	$F_{(1,269)} = 13.87$ $p < 0.01$	$F_{(1,269)} = 6.29$ $p < 0.01$
153	$F_{(1,269)} = 33.85$ $p < 0.01$	$F_{(1,269)} = 19.98$ $p < 0.01$	$F_{(1,269)} = 6.68$ $p < 0.01$
169	$F_{(1,269)} = 5.30$ $p < 0.01$	$F_{(1,269)} = 1.23$ $p > 0.01$	$F_{(1,269)} = 0.31$ $p > 0.01$
170	$F_{(1,269)} = 24.98$ $p < 0.01$	$F_{(1,269)} = 25.72$ $p < 0.01$	$F_{(1,269)} = 8.21$ $p < 0.01$
180	$F_{(1,269)} = 26.31$ $p < 0.01$	$F_{(1,269)} = 28.56$ $p < 0.01$	$F_{(1,269)} = 4.48$ $p < 0.01$

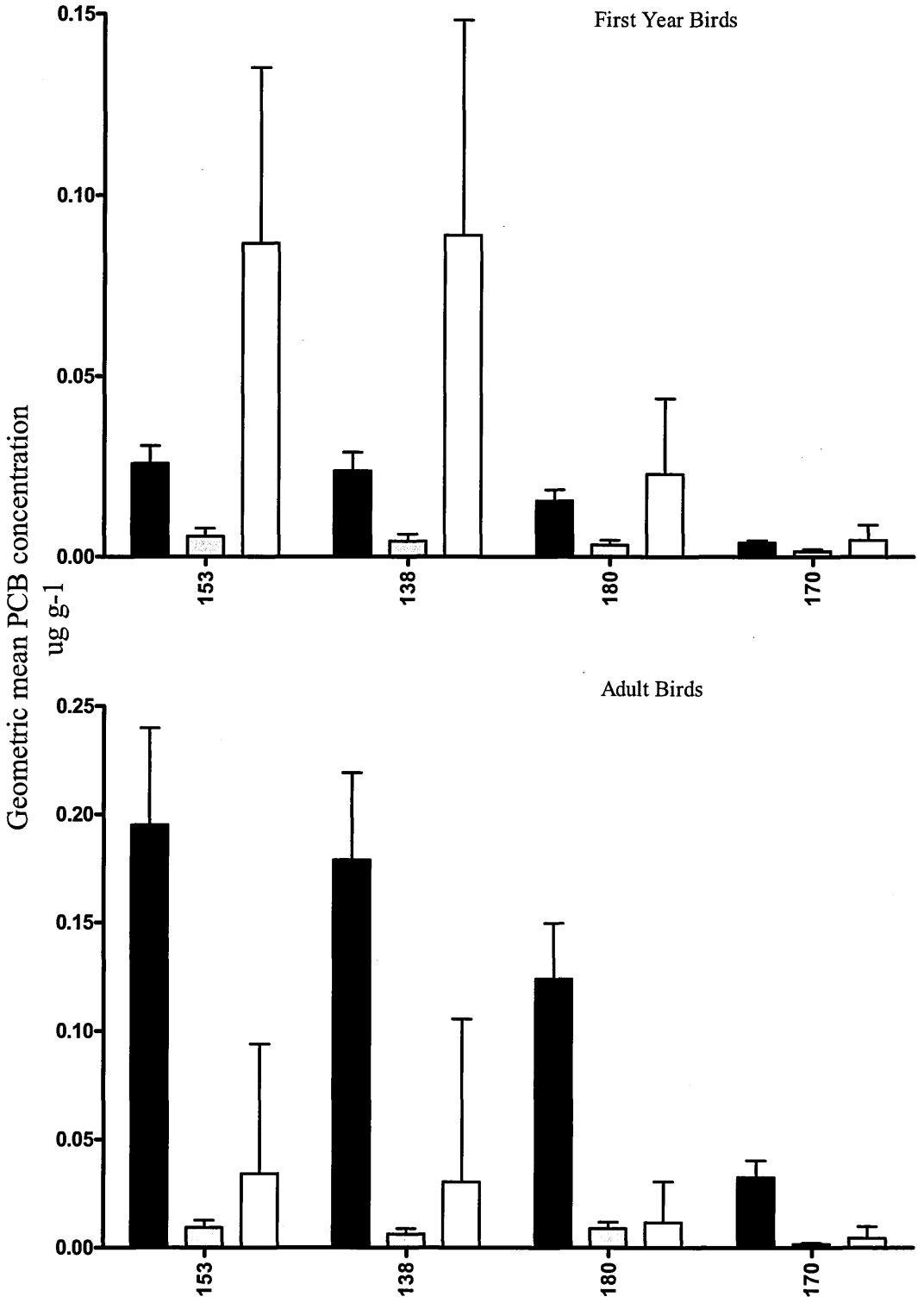

Figure 4.18 Geometric mean (+GSE) liver concentrations (μg g^{-1}) of congeners 153, 138, 180 and 170 in non-starved sparrowhawks (black bars), kestrels (grey bars) and herons (white bars).

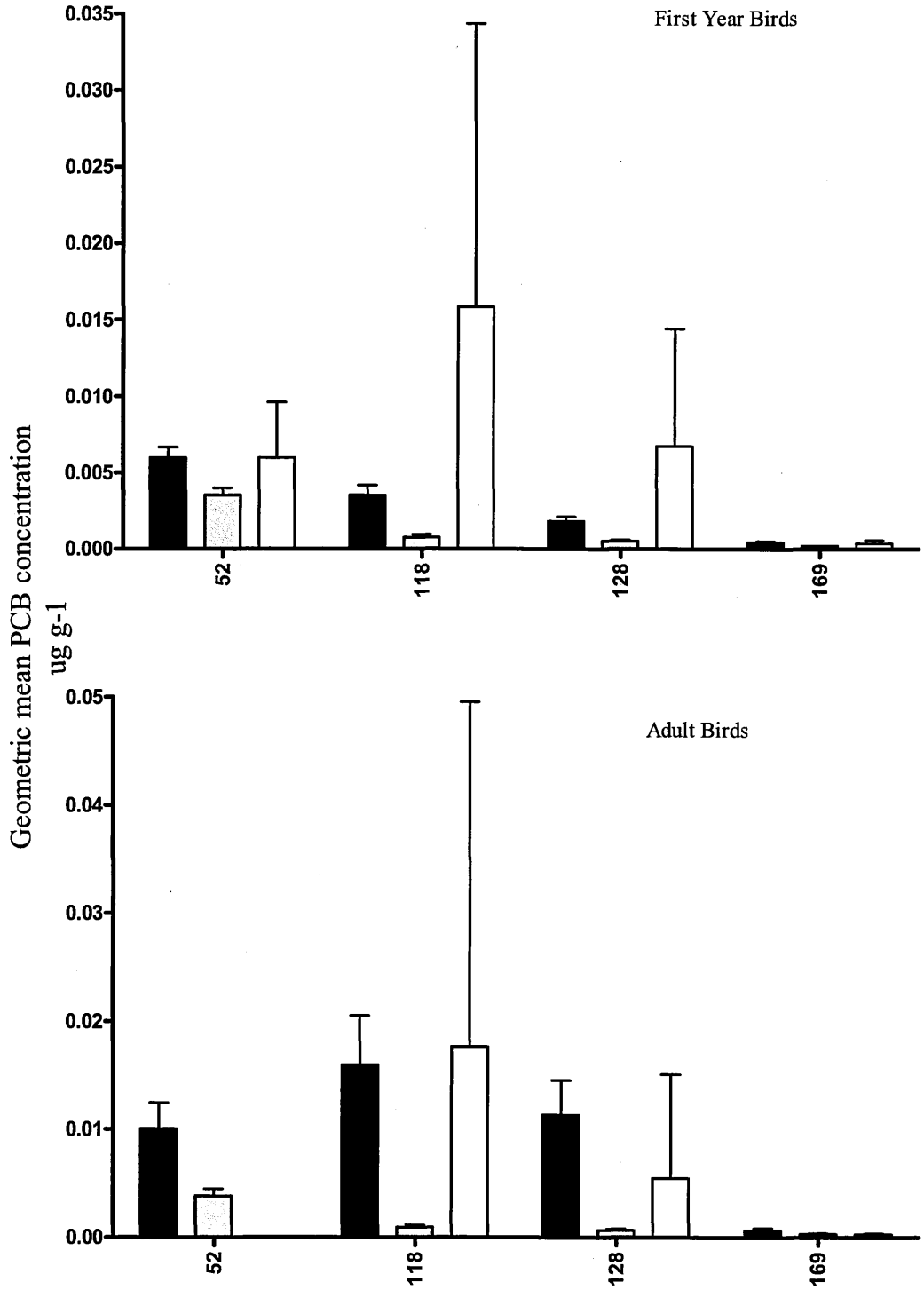

Figure 4.19 Geometric mean (+GSE) liver concentrations (μg g^{-1}) of congeners 52, 118, 128 and 169 in non-starved sparrowhawks (black bars), kestrels (grey bars) and herons (white bars).

In starved birds, species and age both significantly influenced all liver PCB congener concentrations (Table 4.9, Figures 4.20 and 4.21). The differences in mean PCB concentrations between species varied with PCB congener. As with non-starved birds,

starved kestrels had significantly lower liver concentrations of PCBs 52, 118, 128, 149, 169 and 180 than sparrowhawks ($T > 4.40$, $p < 0.005$ in all). Liver concentrations of PCBs 138, 153 and 170 were also slightly lower in starved kestrels than sparrowhawks but these differences were not significant ($T > 2.27$, $p < 0.05$ in all). The differences between kestrels and herons congener concentrations were similar to those in non-starved birds as liver concentrations of PCBs 118, 128, 138 and 153 were significantly lower in kestrels than herons ($T > 3.05$, $p < 0.005$ in all).

Liver concentrations of the other PCB congeners (52, 149, 169, 170 and 180) did not differ between starved kestrels and herons ($T < 2.20$, $p > 0.05$ in all). None of the congeners varied significantly in concentration between sparrowhawks and herons ($T < 1.95$, $p > 0.05$ in all). Unlike in non-starved birds, there were no significant species*age interactions for almost all congeners. Concentrations were typically higher in adult than first-year birds in all three species (Figures 4.20 and 4. 21).

Table 4.9 The results of general linear model analysis of inter-species variation in liver PCB congener concentrations in starved predatory birds.

PCB #	Species	Age	Age*species
52	$F_{(1,316)} = 27.96$ $p < 0.001$	$F_{(1,316)} = 10.39$ $p < 0.001$	$F_{(1,316)} = 0.73$ $p > 0.05$
118	$F_{(1,316)} = 23.61$ $p < 0.001$	$F_{(1,316)} = 30.96$ $p < 0.001$	$F_{(1,316)} = 0.399$ $p > 0.05$
128	$F_{(1,316)} = 8.94$ $p < 0.001$	$F_{(1,316)} = 33.55$ $p < 0.001$	$F_{(1,316)} = 0.707$ $p > 0.05$
138	$F_{(1,316)} = 9.15$ $p < 0.001$	$F_{(1,316)} = 23.58$ $p < 0.001$	$F_{(1,316)} = 0.757$ $p > 0.05$
149	$F_{(1,316)} = 14.02$ $p < 0.001$	$F_{(1,316)} = 9.24$ $p < 0.001$	$F_{(1,316)} = 3.17$ $p < 0.05$
153	$F_{(1,316)} = 5.77$ $p < 0.005$	$F_{(1,316)} = 22.27$ $p < 0.001$	$F_{(1,316)} = 0.860$ $p > 0.05$
169	$F_{(1,316)} = 12.68$ $p < 0.001$	$F_{(1,316)} = 61.64$ $p < 0.001$	$F_{(1,316)} = 0.420$ $p > 0.05$
170	$F_{(1,316)} = 3.78$ $p < 0.05$	$F_{(1,316)} = 30.90$ $p < 0.001$	$F_{(1,316)} = 0.650$ $p > 0.05$
180	$F_{(1,316)} = 4.69$ $p < 0.01$	$F_{(1,316)} = 40.39$ $p < 0.001$	$F_{(1,316)} = 0.557$ $p > 0.05$

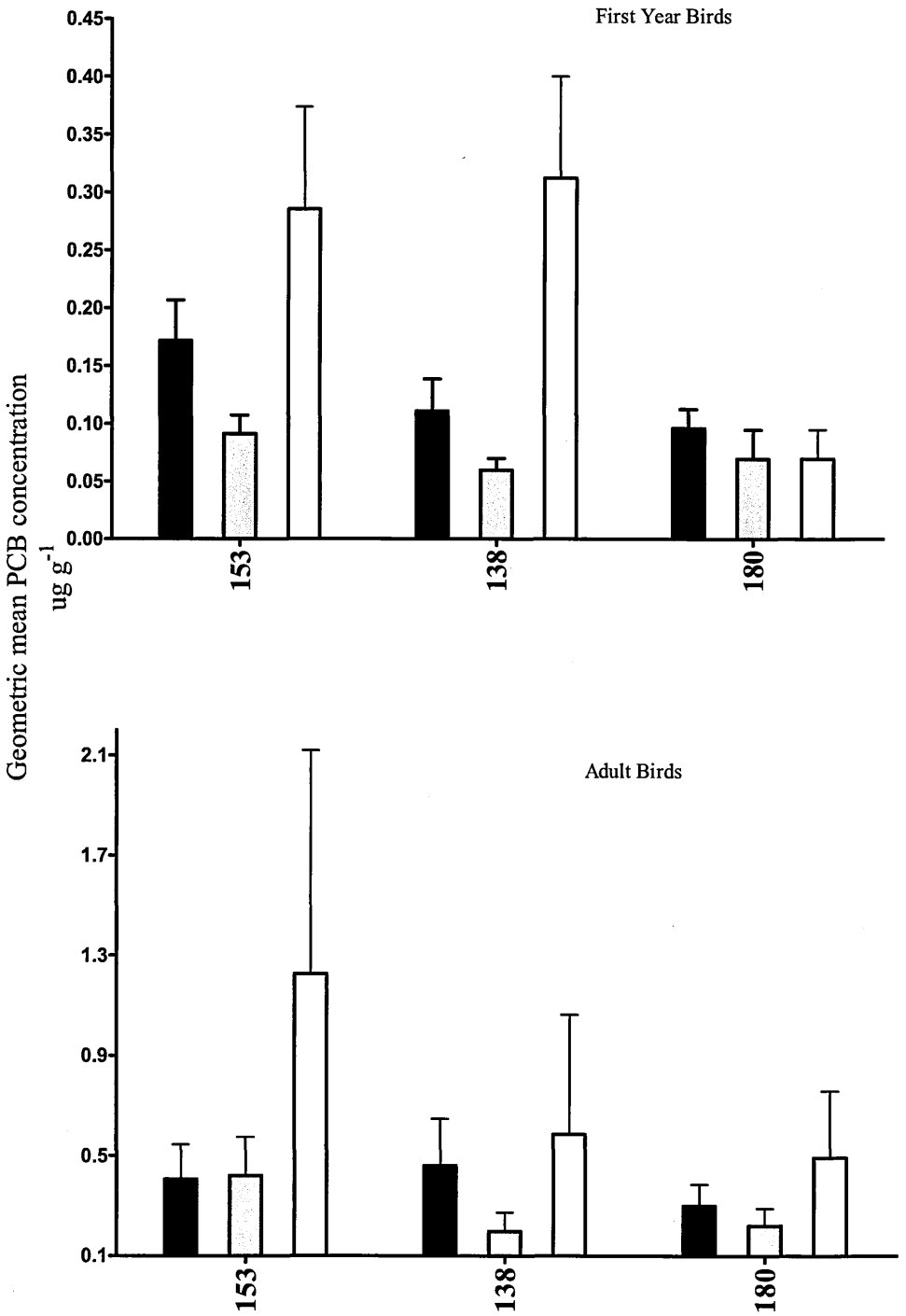

Figure 4.20 Geometric mean (+GSE) liver concentrations ($\mu g\ g^{-1}$) of congeners 153, 138 and 180 in starved sparrowhawks (black bars), kestrels (grey bars) and herons (white bars).

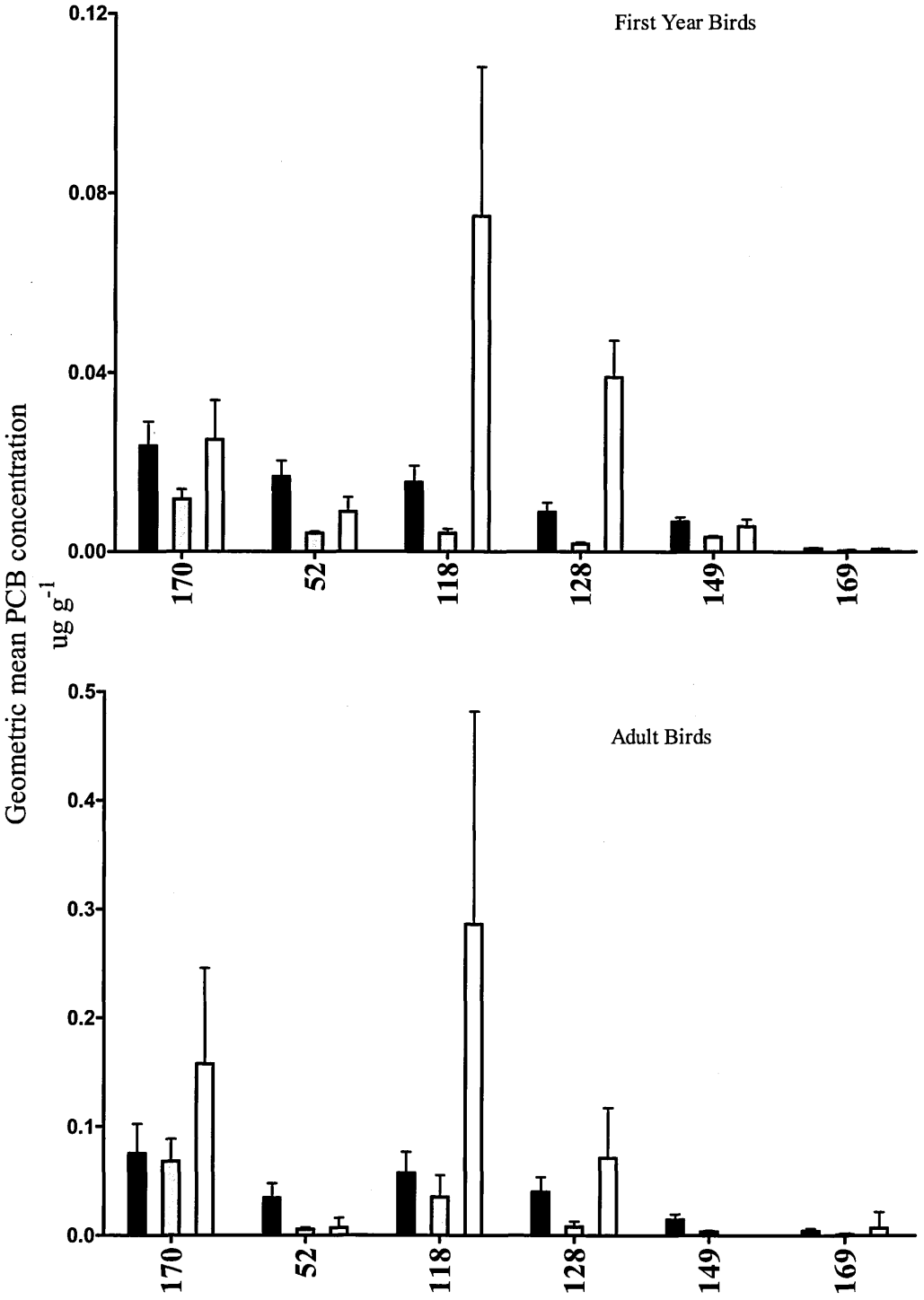

Figure 4.21 Geometric mean (+GSE) liver concentrations (μg g^{-1}) of congeners 170, 52, 118, 128, 149, and 169 in starved sparrowhawks (black bars), kestrels (grey bars) and herons (white bars).

In general, congener profiles were similar between species in that PCBs 153, 138 and 180 consistently accounted for approximately 80% of the summed congener concentrations whilst PCBs 170, 52, 118, 128 and 149 together accounted for between 12 and 20% of liver summed PCB concentrations. Two congeners, PCBs 118 and 128, differed significantly between species in their contribution to the total liver PCB concentrations ($F_{(2,589)} = 54.64$, $p < 0.01$ and $F_{(2,658)} = 53.71$, $p < 0.01$ respectively), accounting for higher proportions of the summed congener concentrations in herons than in either sparrowhawks or kestrels ($T > 6.481$, $p < 0.01$ in all). On average, PCB 118 contributed to 9.3% (± 0.6 SE) of the heron sum PCB concentrations compared to 3.6% (± 0.2 SE) in sparrowhawks and 2.7% (± 0.2 SE) in kestrels. PCB 128 accounted for 4.3% (± 0.5 SE) of the sum PCB concentrations in herons, 2.0% (± 0.1 SE) in sparrowhawks and 1.1% (± 0.1 SE) in kestrels. The percentage contributions of these two congeners also differed significantly between sparrowhawks and kestrels (PCB 118, $T > 3.34$, $p < 0.01$ for both).

4.4 Discussion

The capacity of vertebrates to metabolise individual PCB congeners is influenced by the pattern of chlorine substitution. Hydroxylation tends to occur where there are unsubstituted vicinal *ortho-meta* and *meta-para* positions and PCBs with unsubstituted *para* positions are most readily metabolised (Walker, 2001). PCBs 153 and 180 lack the required unchlorinated phenyl positions whilst PCBs 138 and 170 both have only a single *ortho-meta* position available for metabolism. In raptors, the influence of substitution pattern on PCB congener persistence has been confirmed by laboratory studies on American kestrels (*Falco sparverius)* (Drouillard et al., 2001). The results of the present study also demonstrate that chlorine substitution is a key determinant of the persistence of individual congeners in different predatory bird species. In general, the congener profiles in sparrowhawks, kestrels and herons were related to the potential of

each congener for biotransformation via the hydroxylation of the aromatic rings. Hence, congeners 138, 153, 170 and 180 occurred in the majority of the individuals examined and were bioaccumulated to greater concentrations than other congeners. Similar contamination patterns have been reported either for sparrowhawks, kestrels and herons elsewhere or in other raptors (Kenntner et al., 2003b; Naso et al., 2003; Jenssen et al., 2001; Senthilkumar et al., 2001; Boumphrey et al., 1993). Furthermore, Olafsdottir et al. (2001) reported an increased abundance of the persistent congeners 153, 138 and 180 through the trophic levels of the gyrfalcon (*Falco rusticolus*) food chain reflecting their greater capacity to biomagnify.

Compared to the highly chlorinated congeners, the lower chlorinated congeners have a greater number of unchlorinated ring positions and are therefore more readily metabolised. Hence tri- and tetra-chlorinated congeners were not frequently detected in the birds in the present study. The planar congeners 77, 126, and 169 were also rarely detected in sparrowhawks, kestrels and herons. This may be in part an analytical artefact of the higher limits of detection of these congeners, but may also reflect a higher rate of metabolism of these congeners. Whilst PCBs 77 and 126 both contain unsubstituted *ortho-meta* positions, PCB 169 has none. However Walker (2001) suggested these PCBs are hydroxylated rapidly by the P450 enzyme system via a different pathway because of their planar configuration.

Body condition was probably the most significant influence on the liver concentrations of all the PCB congeners detected in the birds analysed in this study. The influence of body condition on individual congener concentrations was consistent with the findings in Chapter 3 that higher liver total PCB concentrations were associated with starvation. Mean liver concentrations of all the detected congeners were higher in starved than non-starved birds of each species. Whilst total tissue PCB concentrations have been shown to be higher in starved birds (Elliott et al., 1996; Lambeck et al., 1991; Subramanian et

al., 1986), the effect of starvation on individual congeners has only previously been reported in goshawks for a limited range of PCB congeners (Kenntner et al., 2003b). Kenntner et al. (2003b) reported that hepatic concentrations of PCBs 101, 118, 138, 153 and 180 were higher in starved than non-starved goshawks but did not quantify these differences.

The variation in tissue concentrations of lipophilic contaminants such as PCBs with changes in body condition are generally considered to be the consequence of remobilisation of residues from fat. The degree to which PCB congener concentrations were elevated in starved birds varied between individual congeners, and starvation had the largest impact on the more persistent congeners, principally congeners 153, 138 and 180. Because these congeners are not readily metabolised, they are likely to accumulate in fat to higher concentrations than other congeners. Starvation was associated with similar percentage increases in liver concentrations of congeners 118, 128 and 170 (Figure 4.15) but these congeners occurred in much lower concentrations than 153, 138 and 180 (Figs 4.1–4.3), presumably because their partition coefficients between liver and fat are similar to those of congeners 153, 138 and 180, but they are either more readily metabolised, are at lower dietary concentrations, and/or are less readily absorbed across the gut. Starvation was associated with small elevations in concentrations of congeners 52, 101 and 169 compared with the other congeners (Figure 4.15), and this may reflect relatively low partitioning into fat and/or relatively fast metabolism at the time of exposure. Congeners 77, 126, 149 and 169 were rarely detected in non-starved birds but trace levels were found in starved birds. The lack of data for non-starved birds for these congeners means that the effect of body condition on liver concentrations cannot be determined accurately. However, the presence of these congeners in starved sparrowhawks, kestrels and herons confirms that these predatory bird species are either

exposed to these congeners at extremely low concentrations and/or the birds are able to metabolise most of the residues they originally assimilate.

As with total liver PCB concentrations (Chapter 3), age was a second factor which influenced liver concentrations of individual PCB congeners. The effect of age was generally consistent for all congeners. Adult birds had higher liver concentrations than first-year birds, presumably reflecting the longer time-frame over which the adults are exposed to and can assimilate PCBs (Johnstone et al., 1996; Elliott and Shutt, 1993). In kestrels, the difference in mean liver congener concentrations between adults and first-year birds was more pronounced in starved than non-starved birds but this was not the case for sparrowhawks. The reason for this difference between species is uncertain but may be associated with interactions between age-related and species-related differences in metabolic capacity and potentially diet. There was also a surprising, albeit weak, interaction between body condition and age in herons for several congeners in that non-starved adult herons had lower mean liver congener concentrations than first-year herons whilst the opposite was true for starved herons. However, this finding was based on data for only four non-starved adults and may be a statistical anomaly and not representative of the wider population.

In sparrowhawks and kestrels, the liver concentrations of several more persistent congeners (PCBs 118, 138, 153, 170 and 180) differed with sex, as male birds had higher liver congener concentrations than females. The transfer of lipophilic contaminants to eggs is thought to be a major route of elimination for compounds such as PCBs in breeding females (Newton, 1982) and largely account for the sex-related difference in liver PCB concentrations. However, PCB concentrations were also lower in females than males in first-year birds that had not bred. Therefore maternal transfer of contaminants cannot be the only sex-related factor to influence tissue PCB concentrations. It is possible that males and females differ in their dietary intake and

associated assimilation of PCBs and this may partly account for higher liver concentrations in males than females. However, there was also an interaction between body condition and sex, in that the differences in mean congener concentrations between males and females were greater in non-starved than starved birds. During starvation, congener concentrations in female birds increased to levels which were comparable and sometimes slightly greater than those of males. This higher proportional increase in liver congener concentrations as a result of the remobilisation of PCB residues during starvation may indicate that, compared with males, females have a greater capacity to bioaccumulate ingested PCBs into fat. The importance of maternal transfer of PCBs to eggs in accounting for sex-related differences in liver concentrations and in depletion of female body burdens may have been somewhat overestimated.

Once body condition and age were accounted for, there were still significant differences in the magnitude of congener concentrations between sparrowhawks, kestrels and herons. Kestrels had significantly lower liver concentrations of all PCB congeners than either sparrowhawks or herons. This is likely to be due to the higher hepatic activity of their CYP1A enzymes (Walker, 1998; Walker et al., 1987), which are involved in the metabolism of organic contaminants, and/or lower exposure to PCBs through the food-chain. However, which species had the highest liver congener concentrations was dependent on body condition. In non-starved birds, first-year herons had the highest liver congener concentrations whereas in adults, liver concentrations of all congeners were higher in sparrowhawks than either herons or kestrels. This suggests that the way in which dietary exposure, and/or capacity to metabolise persistent organic pollutants, varies with age differs between herons and sparrowhawks. In contrast, although liver congener concentrations varied significantly between species in starved birds, this variation was less pronounced than in non-starved birds. This may be because liver PCB

concentrations in starved individuals are affected by remobilisation of assimilated residues from fat and other tissues, and so may more closely reflect overall lifetime accumulation of PCB residues rather than current exposure. This would imply that differences between species in recent exposure do not result in differences of the same magnitude in assimilation in fat. However, it is also possible that PCB binding sites in the liver are close to saturation in many starved birds and there is comparatively little variation in binding site density between species.

In general the pattern of PCB congeners was similar across the three species examined and in each case was strongly related to the potential of individual congeners to undergo metabolism via the cytochrome P450 enzyme system. The relatively high proportion of herons, compared to sparrowhawks and kestrels, that had detectable concentrations of congener 31 (Figures 4.1–4.3) reflecting the presence of tri-chlorinated PCBs as markers of aquatic PCB exposure as reported by Hoshi et al. (1998). It was notable that the penta- and hexa-chlorinated PCB congeners 118 and 128 contributed to greater proportions of the overall PCB burdens in herons than either sparrowhawks or kestrels, suggesting these congeners may also be indicative of PCB exposure through the aquatic environment.

In conclusion, whilst numerous factors including dietary intake, body condition, age and sex may influence the magnitude of PCB concentrations, the range of different congeners detected in predatory bird livers are most likely to be influenced by the degree of chlorination and substitution pattern and therefore the subsequent tendency of individual congeners to bioaccumulate. The magnitude of PCB congeners was predominantly influenced by body condition and age with individual congeners all affected in a similar manner. However, the extent of these effects on individual congeners varied and this variation was related to the physico-chemical properties of the congeners. The effect of body condition and age on congener concentrations are

therefore likely to influence the toxicity associated with the assimilation of individual congeners, particularly those with dioxin-like configuration and mode of toxicity. How this affects TEQ concentrations in sparrowhawks, kestrels and herons is further explored in Chapter 5.

Chapter Five

PCB Toxic Equivalent Quotients in predatory birds

5.1 Introduction

PCBs have been implicated in a wide range of toxic effects in birds and mammals. Typical PCB-induced responses include adverse effects on reproduction (including embryo mortality and developmental deformities), immuno-suppression, endocrine disruption and hepatotoxicity (Hansen, 1998; Safe, 1993). The non-*ortho* PCBs 77, 81, 126 and 169 together with a group of mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167 and 189) are of particular concern as they have a similar toxic mechanism to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. These compounds have high affinity for the aryl hydrocarbon (A*h*) receptor concerned with the control of biotransformation enzymes involved in the metabolism of hydrocarbon compounds. Interaction with the A*h* receptor results in changes in gene expression and an increase in the level of the hepatic cytochrome P450 enzymes CYP1A1 and CYP1A2. These biochemical effects are considered to underlie many of the harmful effects observed in wildlife (Walker, 2001). The binding affinities of individual congeners to the A*h* receptor vary; however the non-*ortho* congeners are considered the most potent PCBs.

Because of the variation in the potency of different dioxin and dioxin-like compounds and their common mode of action, the Toxic Equivalency Factor (TEF) approach to assessing the likely influence of these compounds on humans and wildlife has been adopted (Van den Berg et al., 1998). This system provides an estimate of both the toxicity and ability to induce CYP1A enzymes of individual A*h* receptor agonists relative to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Individual congeners have been assigned TEF values which indicate the magnitude of response relative to

that of TCDD which has a reference TEF of 1 (Safe, 1993; Van den Berg et al., 1998). These TEF values can then be used in combination with concentration data to determine Toxic Equivalent Quotient (TEQ) concentrations, the TEF values effectively weighting congener concentration data according to its relative potency. TEF values specific to birds, mammals and fish have been compiled (Van den Berg et al., 1998).

The TEF approach has been widely used for assessing the risk from exposure of predatory birds to PCBs and dioxin-like compounds (Merino et al., 2005; Kumar et al., 2002; Sentilkumar et al., 2002; Wiesmuller et al., 2002; Kannan et al., 2001; Zimmerman et al., 1997; Elliott et al., 1996b) and the correlation between increased TEQ concentrations and biochemical response has been documented for several species (Coady et al., 2001; Van den Brink and Bosveld, 2001; Jenssen et al., 2001). Toxicity thresholds for TEQ concentrations in several wild bird species have been proposed and reported lowest observable effect levels (LOEL) show wide inter-species sensitivity ranging from 25 ng g^{-1} lipid weight (lw) in common terns (Bosveld et al., 2000) to 210 ng g^{-1} (lw) in bald eagle chicks (Elliott et al., 1996a) and 2300 ng g^{-1} (lw) in American kestrels (Hoffman et al., 1998). As with PCB concentrations, reported TEQ concentrations are extremely variable both within and between species. Inter-species variation in TEQ concentrations is generally regarded as the result of species differences in metabolism and exposure but the causes of intra-species variation in TEQ concentrations in wildlife have not previously been determined.

The aims of this chapter are to report ∑TEQ concentrations for sparrowhawks, kestrels and herons from the UK and to determine the main causes of intra-species variation in ∑TEQ concentrations in birds. Body condition, age and sex have all been shown to be important in explaining much of the variation in liver PCB concentrations (Chapters 3 and 4) and would therefore be expected to have a similar influence on TEQ concentrations. Finally, TEQ concentrations are compared between the three species.

5.2 Methods

Four dioxin-like PCBs were analysed as part of this study. They were the only PCBs that were quantified by the PBMS for which Toxic Equivalent Factors (TEF) were available. The full suite of PCB congeners for which TEFs have been published were not included as part of the routine PBMS monitoring until 1998. The dioxin-like congeners quantified in this study were PCBs 118, 77, 126 and 169; the latter three are the most toxic co-planar PCBs to birds as identified by Van den Berg et al. (1998).

TEQ concentrations were calculated for each individual congener by multiplying the congener WHO TEF value (Table 5.1) by the congener concentration. TEQs are expressed as pg g^{-1} wet weight.

Table 5.1 TEF values for dioxin-like PCB congeners.

PCB Congener	*TEF Value*
77	0.05
118	0.00001
126	0.1
169	0.001

Total ($\sum$) TEQ values were calculated by summing the individual congener TEQ concentrations for each liver sample. For samples that had no detectable $\sum$TEQ concentrations, a value of half the lowest $\sum$TEQ concentration (0.0025 pg g^{-1}) was assigned. All statistical analysis was carried out on $\log_{10}$ transformed data to ensure data conformed to the underlying assumptions necessary for statistical models.

5.2.1 Intra-species variation in liver TEQ concentrations

∑TEQ concentrations for each species were initially examined by Pearson product moment correlation to determine whether they were correlated with ∑PCB concentrations.

As with PCB concentrations (Chapters 3 and 4), the TEQ concentrations were analysed by a backwards stepwise adjusted general linear model (GLM) to determine sources of intra-species variation. Body condition, age and sex were entered into the model along with their respective interaction terms. Age and sex were treated as co-variables. Following analysis of the full model, the term that explained the least amount of variation and was not statistically significant was removed from the model. The analysis was then repeated successively until the only terms remaining were those that were statistically significant ($p < 0.01$). The data for sparrowhawks, kestrels and herons were analysed separately.

5.2.2 Inter-species variation in liver TEQ concentrations

∑TEQ concentrations were analysed by sequential general linear model (GLM) analysis. Body condition, age, species and their respective interaction terms were entered into the model in that order. Age was treated as a co-variable.

5.3 Results

Overall, the geometric mean (± GSE range) ∑TEQ concentrations were 0.39 (0.35–0.43) pg g^{-1} in sparrowhawks, 0.03 (0.02–0.04) pg g^{-1} in kestrels and 1.17 (0.74–1.85) pg g^{-1} in herons.

∑TEQ concentrations were positively correlated with ∑PCB concentrations in all three species ($r = 0.68$ in sparrowhawks, $r = 0.63$ in kestrels and $r = 0.70$ in herons, $p < 0.01$ for all) with TEQ concentrations clustered according to the number of individual

congeners contributing to the ∑TEQ value (Figures 5.1–5.3). Where only one congener accounted for the overall liver TEQ concentration in sparrowhawks, kestrels and herons this was entirely due to the presence of PCB 118. In sparrowhawks, the higher TEQ concentrations were associated with the presence of two and three dioxin-like PCBs whilst the TEQ concentrations in kestrels and herons were influenced by at most two of the dioxin-like congeners.

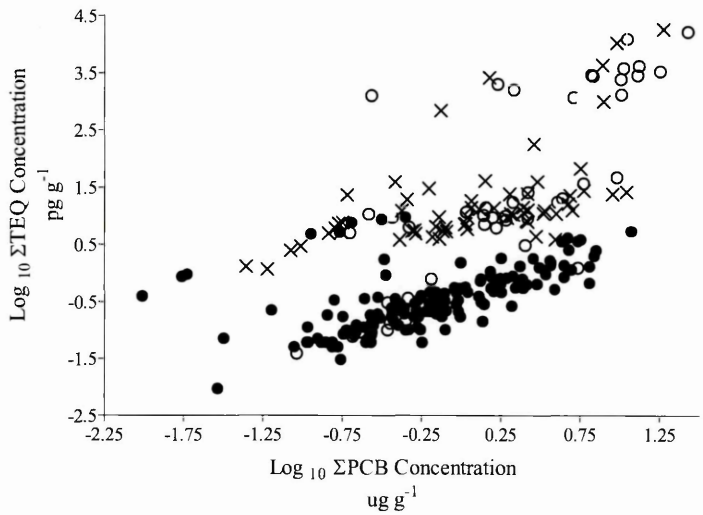

Figure 5.1 ∑TEQ concentrations in sparrowhawks. Symbols denote the number of congeners contributing to the overall TEQ value (closed circles = 1 congener, crosses = 2 congeners, filled squares = 3 congeners and open circles = 4 congeners).

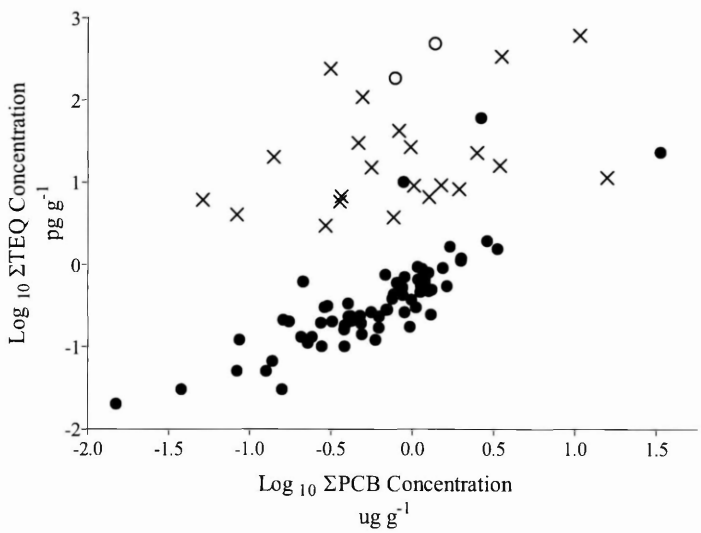

Figure 5.2 ∑TEQ concentrations in kestrels. Symbols denote the number of congeners contributing to the overall TEQ value (closed circles = 1 congener, crosses = 2 congeners, filled squares = 3 congeners and open circles = 4 congeners).

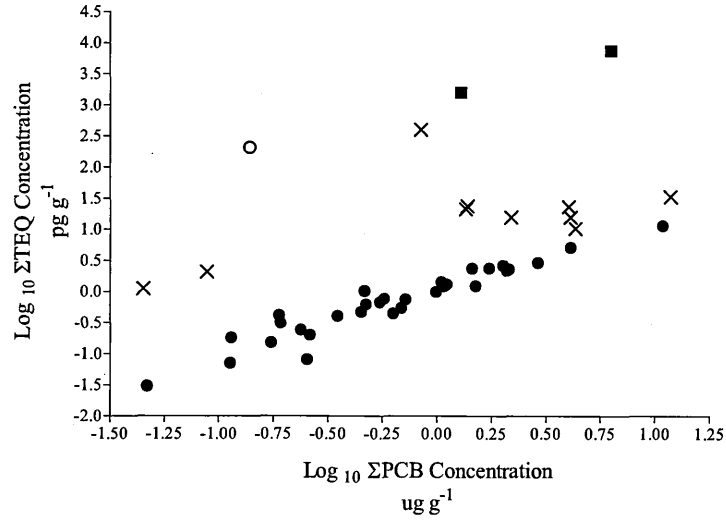

Figure 5.3 ∑TEQ concentrations in herons. Symbols denote the number of congeners contributing to the overall TEQ value (closed circles = 1 congener, crosses = 2 congeners, filled squares = 3 congeners and open circles = 4 congeners).

5.3.1 Intra-species variation in liver TEQ concentrations

In sparrowhawks, ∑TEQ concentrations varied significantly with body condition ($F_{(1,347)} = 22.29$, $p < 0.01$) and age ($F_{(1,347)} = 22.55$, $p < 0.01$). TEQ concentrations in starved birds were up to 16 times higher than in non-starved birds. Adult sparrowhawks had higher TEQ concentrations than first-year birds (Figure 5.4). In non-starved adult

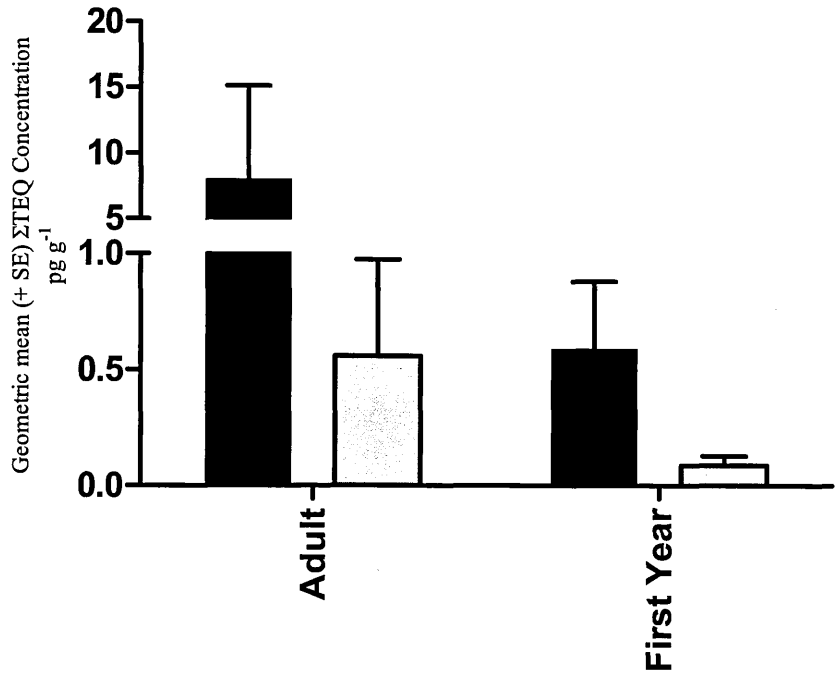

Figure 5.4 Intra-species variation in liver ∑TEQ concentrations in starved (black bars) and non-starved (grey bars) sparrowhawks.

sparrowhawks PCB 118 contributed to the bulk of the TEQ burden accounting for 67% of the mean ∑TEQ with PCBs 169 and 77 contributing equally to a further 30% (Figure 5.5). In contrast, PCB 169 was the major component of ∑TEQ concentrations in starved adult birds and it accounted for over 60% of the mean ∑TEQ.

The TEQ profile of first-year birds was similar between non-starved and starved sparrowhawks with PCBs occurring in the order 118 > 169 >77 >126 (Figure 5.5).

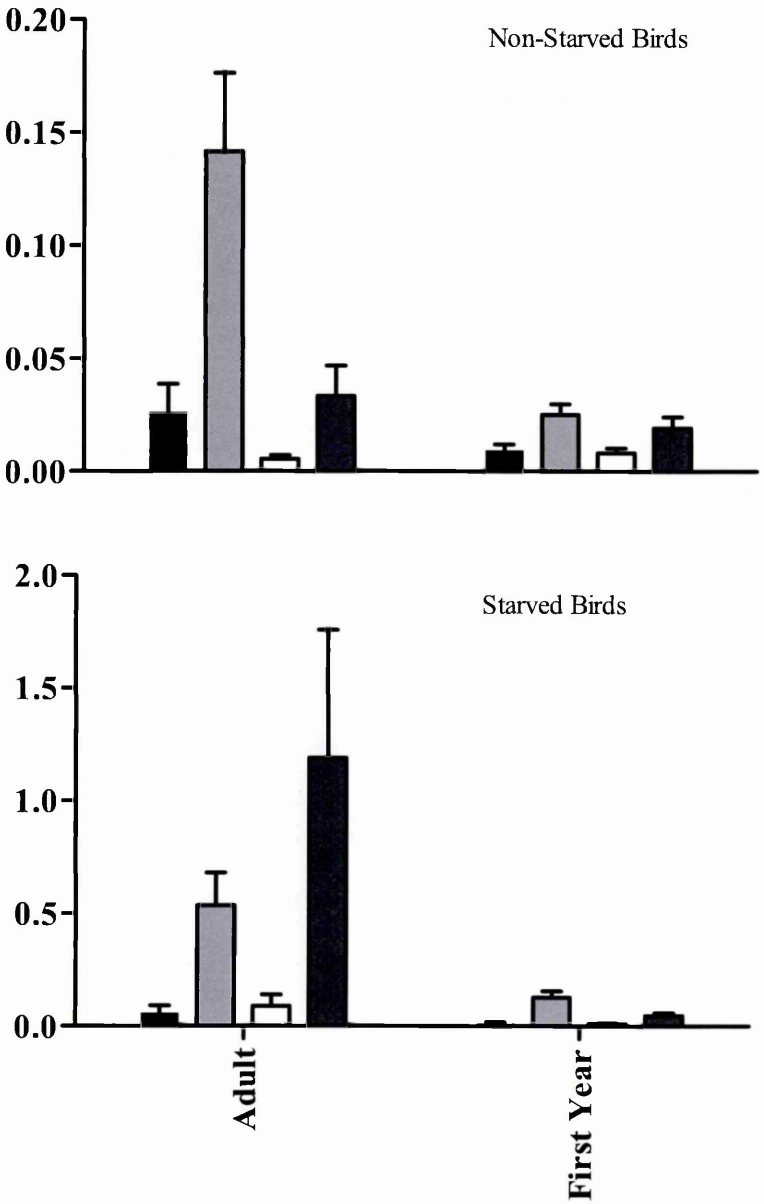

Figure 5.5 Congener specific TEQ concentrations in sparrowhawks (PCB 77 = black bars, PCB 118 = light grey bars, PCB 126 = white bars, PCB 169 = dark grey bars).

In kestrels, liver TEQ concentrations were also significantly higher in starved than non-starved kestrels ($F_{(1,193)} = 28.51$, $p < 0.01$) and in adults compared with first-years ($F_{(1,193)} = 19.77$, $p < 0.01$). Furthermore, there was a significant interaction between body condition and age ($F_{(1,193)} = 7.70$, $p < 0.01$) in that the difference between adult and first-year birds was much greater in starved than non-starved kestrels (Figure 5.6). This in fact also seemed to be the case in sparrowhawks (Figure 5.5) but the interaction term did not achieve statistical significance.

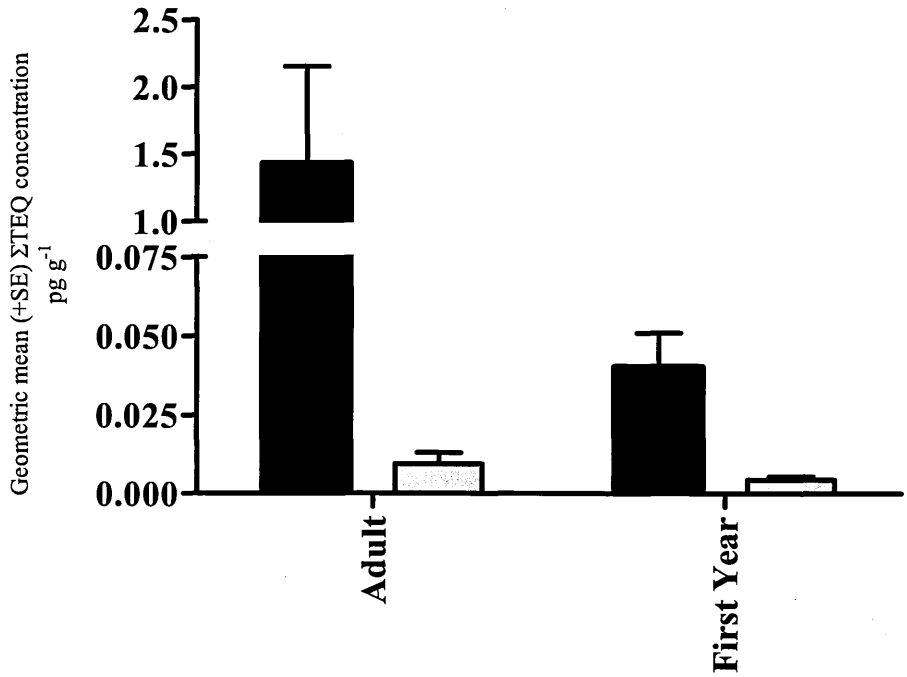

Figure 5.6 Intra-species variation in liver ∑TEQ concentrations in starved (black bars) and non-starved (grey bars) kestrels.

∑TEQ concentrations in non-starved kestrels were solely due to PCBs 118 and 169 which contributed in both adult and first-year birds (Figure 5.7). In starved kestrels PCB 118 explained 70% and 75% of the ∑TEQ concentrations in first-year and adult birds respectively. As in non-starved adults, PCB 169 was the only other congener contributing to ∑TEQs in starved kestrels (as found in non-starved adults). However, PCB 77 was also detected in starved first-year birds and PCBs 77 and 169 accounted for similar proportions of the ∑TEQ concentration (Figure 5.7).

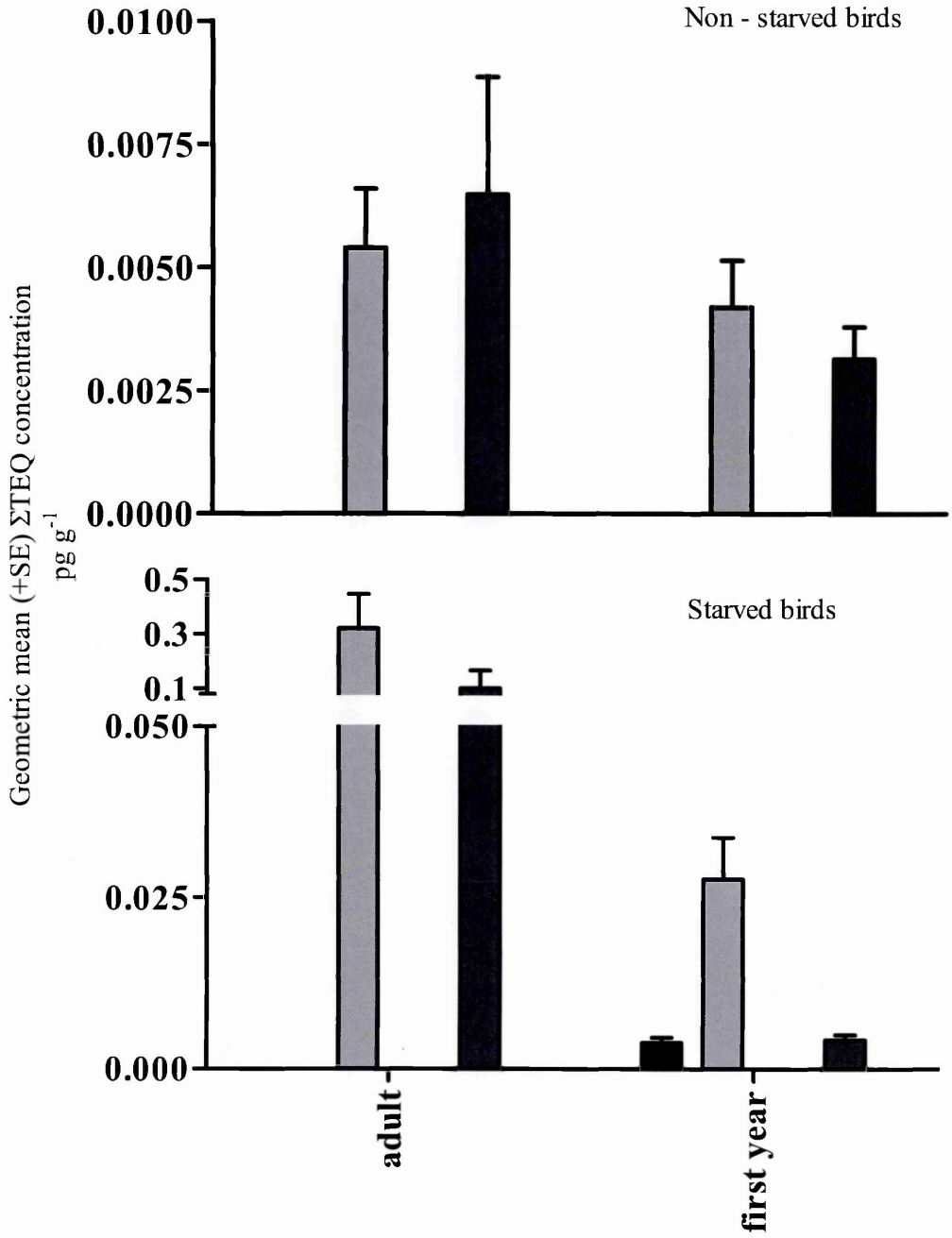

Figure 5.7 Congener specific TEQ concentrations in kestrels (PCB 77 = black bars, PCB 118 = light grey bars, PCB 169 = dark grey bars).

Body condition was the only statistically significant factor to influence ∑TEQ concentrations in herons ($F_{(1,45)} = 5.98$, $p < 0.05$). ∑TEQ concentrations were tenfold higher in starved than in non-starved birds (Figure 5.8). As with sparrowhawks and kestrels, TEQ concentrations tended to be higher in adult than first-year birds, particularly in starved individuals (Figure 5.10), but the effect of age was not

statistically significant. PCB 118 accounted for over 90% of the ∑TEQ concentrations in both starved and non-starved herons (Figure 5.9). In non-starved birds PCB 169 was the only other congener present, whereas in starved herons the congener specific TEQ concentrations occurred in the order PCB 169 > 77 > 126.

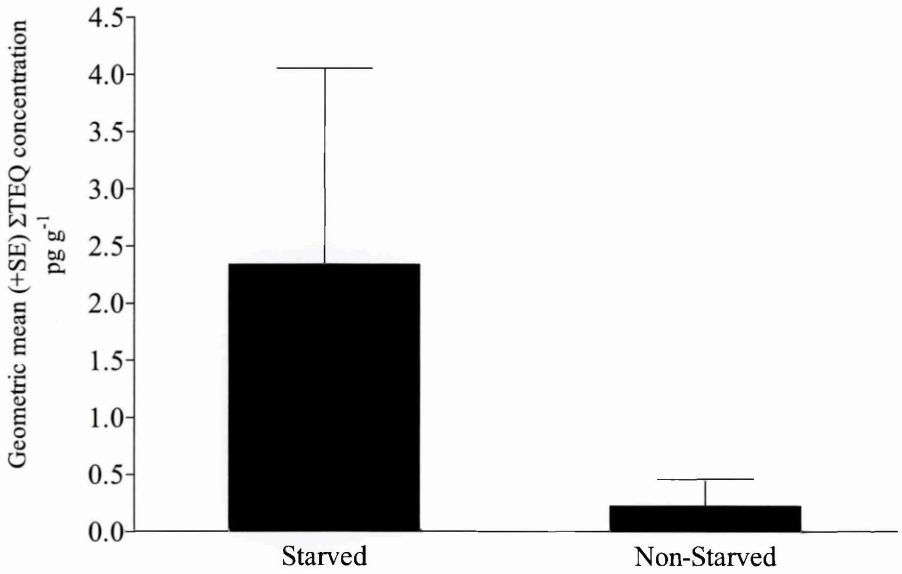

Figure 5.8 Intra-species variation in liver ∑TEQ concentrations in herons.

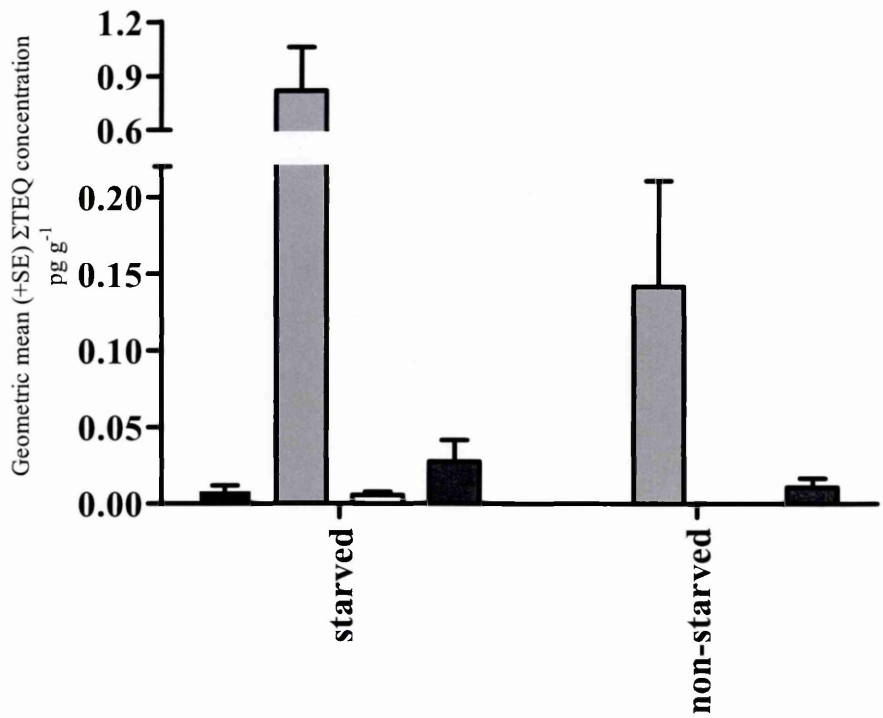

Figure 5.9 Congener specific TEQ concentrations in herons (PCB 77 = black bars, PCB 118 = light grey bars, PCB 169 = dark grey bars).

5.3.2 Inter-species variation in liver TEQ concentrations

Liver ∑TEQ concentrations varied significantly between species ($F_{(2,586)} = 50.06$, $p < 0.01$). Post hoc analysis by Tukeys pairwise comparison confirmed that kestrels had significantly lower ∑TEQ concentrations than sparrowhawks ($T = -5.62$, $p < 0.01$, Figure 5.10). Mean ∑TEQ concentrations did not differ significantly between herons and either sparrowhawks or kestrels ($T < 2.71$, $p > 0.01$ in both).

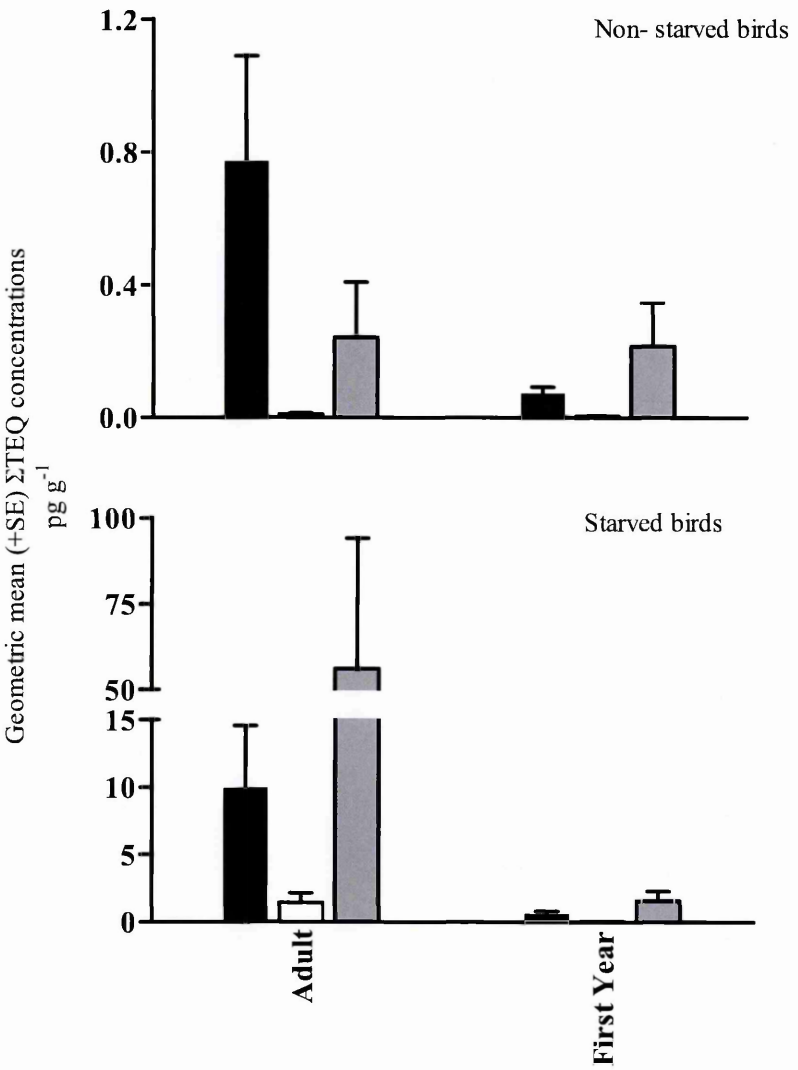

Figure 5.10 ∑TEQ concentrations in sparrowhawks (black bars), kestrels (white bars) and herons (grey bars).

However, mean TEQ concentrations in herons were up to 40 times those of kestrels (Figure 5.10) but there was wide variation in TEQ concentrations between individuals

and the power of the statistical comparisons may have been limited by this and by the relatively small sample size, particularly for herons.

Consistent with the results of the previous intra-species variation analysis, both body condition and age significantly influenced ∑TEQ concentrations ($F_{(1,586)} = 37.52$ and 50.06 respectively, $p < 0.01$ in both). None of the interaction terms were statistically significant.

5.4 Discussion

Whilst ∑TEQ concentrations have been reported for tissues and eggs in numerous raptor and fish-eating bird species elsewhere (Merino et al., 2005; Senthilkumar et al., 2002; Kumar et al., 2002; Wiesmuller et al., 2002; Jenssen et al., 2001; Kannan et al., 2001; Guruge et al., 2000; Elliott et al., 1996b), similar data for the UK are sparse; studies have been limited to relatively few samples and represent localised sampling areas (Thompson et al., 2003; Boumphrey et al., 1993). The present study is the first comprehensive report of TEQ concentrations quantified in predatory birds collected from throughout the UK.

Mean TEQ concentrations vary widely both within and between individual studies, and range from13 pg g^{-1} wet weight (Merino et al., 2005) to over 2700 µg g^{-1} wet weight (Elliott et al., 1996b). Direct comparison of the ∑TEQ concentrations quantified as part of this study with other reported values is not possible as other studies calculate ∑TEQ concentrations for a wider range of both PCBs and dioxin compounds, although the non-*ortho* PCBs 77, 81, 126 and 169 typically contributed to 45–88% of the overall TEQ concentration in those studies (Merino et al., 2005; Senthilkumar et al., 2002; Wiesmuller et al., 2002; Kannan et al., 2001). The non-*ortho* PCB ∑TEQ concentrations in British sparrowhawks, kestrels and herons were notably lower than concentrations reported elsewhere, although the results for starved herons were of a

similar magnitude. They were also lower than those reported for nestling herons from a single heronry in the UK (Thompson et al., 2003). Thus it is likely that the ∑TEQ concentrations reported in the present study are underestimates of actual TEQ concentrations to some extent. However, differences between the ∑TEQ concentrations in British sparrowhawks, kestrels and herons in the present study and those in predatory birds elsewhere may not solely be due to variation in the number of dioxin-like compounds that were determined. It was notable that congeners 118 and 169 accounted for the greater part of liver ∑TEQ concentrations in the British birds of prey whereas PCBs 77 and 126 were the major contributors to TEQ concentrations in other raptors (Merino et al., 2005; Senthilkumar et al., 2002; Wiesmuller et al., 2002; Elliott et al., 1996b) and fish-eating birds (Senthilkumar et al., 2002; Guruge et al., 2000; Jenssen et al., 2001). PCBs 77 and 126 were determined but rarely detected in the present study. Both congeners have high TEFs (Table 5.1) and therefore assimilation of even relatively low concentrations markedly elevates liver ∑TEQ concentrations. Thus the results suggest that lower ∑TEQ concentrations in sparrowhawks, kestrels and herons in Britain compared with concentrations in similar species from mainland Europe and North America may partly reflect a lower exposure to dioxin-like compounds.

The high intra-species variability in ∑TEQ concentrations observed in sparrowhawks, kestrels and herons found in this study is consistent with the findings of others (Kumar et al., 2002; Jenssen et al., 2001; Guruge et al., 2000; Elliott et al., 1996b). Unlike the other studies, the present study quantified the importance of body condition and age in explaining this high degree of variation. Body condition was the most important factor and the inverse relationship between fat score and ∑TEQ concentrations reflected the influence starvation had generally on liver PCB concentrations (Chapter 4). The remobilisation of the dioxin-like PCBs from fat during starvation consequently raised overall TEQ concentrations in starved birds, and the weightings associated with

individual congener TEF values resulted in a disproportionate rise in ∑TEQ concentrations between starved and non-starved birds compared to the increase in individual congener concentrations alone.

Liver TEQ concentrations were also influenced by age in a manner similar to that reported for PCB congener concentrations (Chapter 4) with adults having higher TEQ concentrations than first-year birds as TEQ concentrations reflected the age-related accumulation of congeners 118 and 169 reported in Chapter 4. Higher TEQ values in adults than chicks were likewise reported for cormorants (*Phalacrocorax carbo*) compared to chicks (Guruge et al, 2000), and Jones et al. (1994) demonstrated increasing accumulation rates of TEQ concentrations in cormorant chicks from the Great Lakes.

Of the sparrowhawks, kestrels and herons, the highest mean ∑TEQ concentration was in starved adult herons (Figure 5.10) and was approximately equivalent to a lipid weight concentration of 3 ng g^{-1} assuming a wet to lipid conversion factor of approximately 30. This was some two to three orders of magnitude below reported lower observable effect concentration (LOEC) in eggs of 210 ng g^{-1} (lw) for bald eagles (*Haliaeetus leucocephalus*) (Elliott et al., 1996a) and 2300 ng g^{-1} (lw) for American kestrels (*Falco sparverius*) (Hoffman et al., 1998) and an order of magnitude lower than the liver LOEC of 25 ng kg^{-1} (lw) reported for CYP1A1 induction in common terns (*Sterna hirundo*) (Bosveld et al., 2000). Therefore, although the ∑TEQ concentrations reported in the present study for sparrowhawks, kestrels and herons may be underestimated to some extent; it still remains unlikely that average ∑TEQ concentrations in these species in Britain are at toxicologically significant levels. This is particularly true for kestrels as they had the lowest ∑TEQ concentrations of the three species, which may reflect lower exposure and greater ability to metabolise dioxin like PCBs.

Whilst TEQ levels were relatively low overall in each species compared to those in birds reported elsewhere, my results have confirmed the importance of intrinsic physiological factors such as body condition and age in determining liver TEQ concentrations in birds. This may equally well apply to other vertebrates. These results would indicate that in order to more accurately assess the risk of wildlife from dioxin-like compounds using the TEF approach, factors such as body condition and age should be accounted for in future studies. This is of particular significance as a recent re-evaluation of the TEF system discussed widening the scope of the TEF approach to include several other classes of dioxin-like compounds and persistent organochlorine pollutants such as polybrominated biphenyls and polychlorinated naphthalenes (Van den Berg et al., 2006), all of which have the potential to be influenced by differences in nutritional state, age and sex in a similar manner to that observed for PCBs.

Chapter Six

Reassessing temporal PCB trends

6.1 Introduction

The Predatory Bird Monitoring Scheme (PBMS) has monitored total PCB concentrations in both eggs and livers of several predatory bird species since the mid-1960s. Following restrictions in the production and in the use of PCBs, the concentrations of these compounds in the eggs of various predatory bird species and in heron livers have declined significantly (Shore et al., 2005). However, PCB residues in sparrowhawk livers show only a slight (non-significant) downward trend, whilst in kestrels there has been no evidence of any progressive decline in liver PCBs since monitoring began (Shore et al., 2005). Other studies on terrestrial raptor species have similarly reported little change in tissue PCB concentrations over time (Kenntner, 2003a; Johnstone, 1996; Jarman, 1993), whilst tissue PCB residues in other avian species have declined slowly with concentrations typically being highly variable between individuals (Olafsdottir et al., 2005; Thyen et al., 2000).

The analysis described in Chapter 3 demonstrated that body condition, age, and sex all significantly affected the magnitude of liver PCB concentrations in sparrowhawks, kestrels and herons collected through the PBMS during the 1990s. Body condition was by far the most important factor and explained up to 40% of the variation in liver PCB concentrations (Wienburg and Shore, 2004). The effect of body condition on liver residue is likely to be due to remobilisation of PCBs from fat as birds utilise their fat stores during periods of high energetic demand such as breeding, moulting and migration or during periods when prey are scarce and energy intake fails to match expenditure. However the long-term monitoring of PCB concentrations conducted by

the PBMS and in other studies has not taken into account any physiological factors which may influence the concentrations measured. Given the importance of body condition in determining liver PCB congener concentrations any variation between years in the proportions of birds that were in poor condition (and may have metabolised their fat reserves) may have introduced considerable variation into the long-term liver total PCB data. Such variation may therefore obscure long-term changes in liver PCB concentrations that are due to a reduction in dietary PCB intake or the rate of those changes.

The main aim of the study described in this chapter was to determine whether taking nutritional state into account affected the detection of long-term changes in liver concentrations of PCBs in sparrowhawks, kestrels and herons. A secondary aim was to examine whether any variation in liver PCBs with nutritional state was indeed caused by remobilisation of residues from fat, or might simply be due to organ wastage associated with starvation.

6.2 Methods

Congener-specific PCB concentrations have only been quantified by the PBMS since 1992. Therefore in order to assess the impact of nutritional state on the long-term changes in liver PCBs, archived data for total PCB concentrations measured between 1965 and 2003 were used.

6.2.1 Chemical analysis

All samples were extracted using the extraction and cleanup methods outlined in Chapter 2. However, as with any long-term monitoring program, there have been minor methodological and instrumental changes over time. Full method and quality assurance details covering the span of the PBMS program are given in Wienburg and Shore (2004), Newton et al. (1993) and Newton and Bogan (1979).

Total PCB concentrations were calculated as the sum area of all peaks appearing in the sample chromatogram which could not be identified as one of six organochlorine pesticides routinely quantified by the PBMS. PCB residues were quantified by comparison of the total sample PCB peak area with the sum peak area of an Arochlor 1254 calibration standard of 4 $\mu g\ ml^{-1}$ concentration. To allow comparability with earlier analytical results, the limits of detection for all samples analysed were taken as 0.01 $\mu g\ g^{-1}$ wet weight. This was the limit of detection recorded with the analytical data during the first 15 years of the monitoring scheme.

6.2.2 Data analysis

Data for each species were analysed separately. Concentration data were $\log_{10}$ transformed so that the underlying assumptions of the statistical models were met. To determine overall temporal trends for liver PCB concentrations the data were initially analysed by weighted linear regression, the geometric mean liver PCB concentration in each year was the response variable and year the predictor variable. In herons, data for each three-year period were combined to ensure sufficient sample numbers for data analysis. Birds were then allocated to one of two groups – birds that had been diagnosed as having died through starvation and birds that had died from other causes (non-starved birds). The annual geometric mean liver PCB concentrations were then calculated for starved and non-starved birds in each year. To determine whether time and body condition significantly influenced liver PCB concentrations, data were analysed by sequential type (I) general linear model (GLM) analysis with year as a covariate. Body condition was the first factor fitted in the GLM model followed by year and then the interaction term (body condition*year). When time was found to be statistically significant in the model, the data were subsequently analysed using multiple regression analysis with year and starvation category as the predictor variables. This was done to examine in more detail the nature of any change in liver PCB concentrations over time.

Although non-starved birds had not died as a direct result of starvation some individuals may also have been in poor body condition at the time of death and already have metabolised much of their fat reserves. Thus separating birds into starved and non-starved categories for this analysis was not a perfect way to determine the effect of nutritional state on long-term trends in liver PCB concentrations. Ideally birds would have been separated by their fat score values as in Chapter 3 but fat scores were not recorded in the PBMS post-mortems until 1992. The imperfect distribution of starved and non-starved birds meant that any progressive change in the proportion of non-starved birds with low fat levels might unduly influence temporal trends in liver contaminant concentrations within this group. The proportion of non-starved birds with fat scores of 0 or 1 (corresponding to a starved condition) in each year between 1992 and 2003 was determined. Any change in this proportion over time was examined by regression analysis.

Finally, to examine the extent to which the changes in liver PCB concentration associated with starvation were explained by organ wastage or remobilisation from fat stores, the difference in liver weights between starved (fat score 0 and 1) and non-starved (fat score 2–5) were analysed by 2-tailed student t test.

To determine whether difference in liver PCB concentrations between starved and non-starved birds could be explained by changes in liver weight alone the differences in annual geometric mean liver PCB concentrations and annual mean liver weights between starved and non-starved birds were compared using a paired t test with year as the replicate to determine whether these values were significantly different.

6.3 Results

A total of 1944 sparrowhawks, 1283 kestrels and 701 herons received in the period between 1963 and 2003 were analysed.

Table 6.1 The number of bird carcasses collected during each decade of the PBMS.

Years	Sparrowhawk			Kestrel			Heron		
	Starved	Non-Starved	Total	Starved	Non-Starved	Total	Starved	Non-Starved	Total
1964–1973	6	93	99	88	147	235	22	121	143
1974–1983	88	338	426	181	256	437	65	120	185
1984–1993	236	597	833	260	220	480	96	202	298
1994–2003	156	430	586	63	68	131	29	46	75

6.3.1 Temporal trends in liver PCB concentrations

Total PCBs declined significantly in herons during the monitoring period when analysed by GLM ($R^2 = 0.763$, $F_{(1,10)} = 32.17$, $p < 0.001$, Figure 6.1). Time and body condition were both found to explain variation in liver PCB residues ($F_{(1,20)} = 31.62$ and $F_{(1,20)} = 47.64$ respectively, $p < 0.001$ in both cases). Concentrations were higher in starved herons than in non-starved herons (Figure 6.2). The decline in liver residues over time was more marked in non-starved birds but the difference was not statistically significant.

When the data for all (starved and non-starved) sparrowhawks were analysed together, there was no significant change over time in liver PCB concentrations ($R^2 = 0.35$, $F = 1.02$, $p > 0.01$) (Figure 6.3). When body condition and time were included in the GLM analysis, body condition was by far the most important factor in determining liver PCB concentrations ($F_{(1,20)} = 30.34$, $p < 0.001$), and explained 58% of the variation in residue levels. As previously shown in Chapter 3, liver PCB concentrations were higher in starved than non-starved sparrowhawks. Time also became a significant, albeit weak,

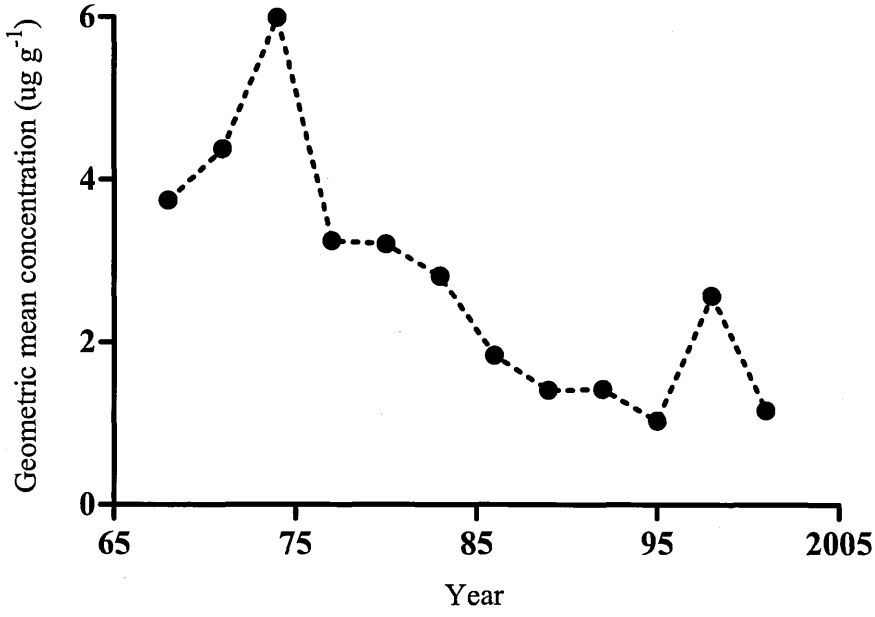

Figure 6.1 Annual mean total PCB concentrations in heron livers analysed between 1967 and 2003. Data for pairs of years were combined to give adequate sample sizes for analysis. The numbers of birds in each 'pair of years' were between x and y.

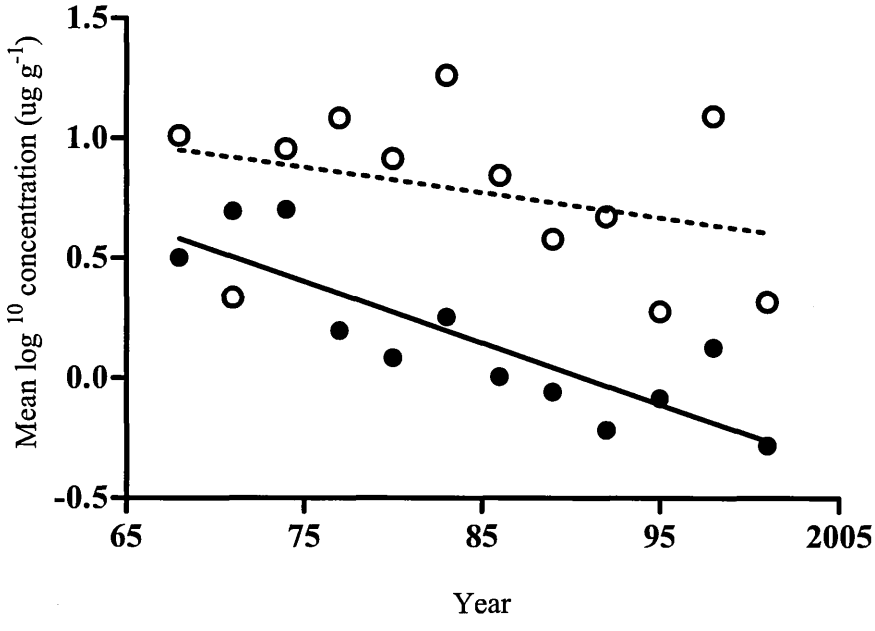

Figure 6.2 Annual mean total PCB concentrations in starved (open circles) and non-starved (filled circles) heron livers. Data for pairs of years were combined to give adequate sample sizes for analysis. The numbers of birds in each 'pair of years' were between x and y.

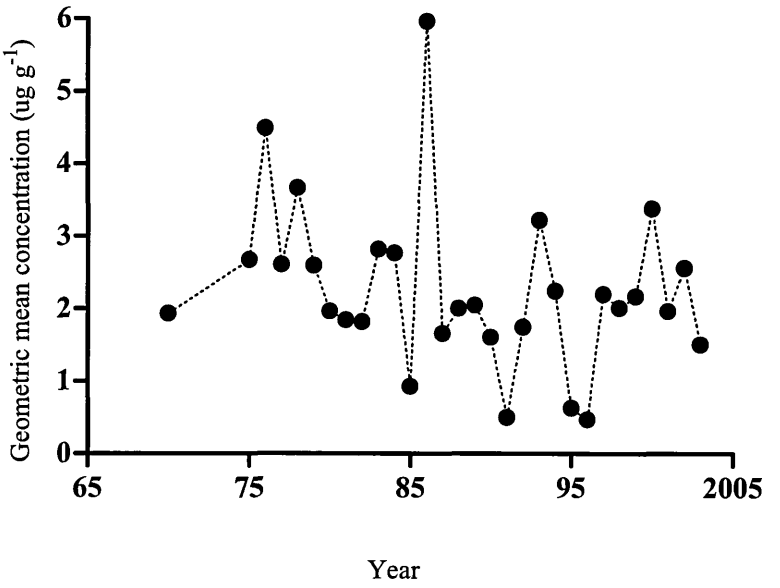

Figure 6.3 Annual mean total PCB concentrations in sparrowhawk livers analysed between 1967 and 2003.

influence ($F_{(1,20)} = 5.49$, $p < 0.05$). However, when the time trend for liver residues were analysed by weighted linear regression for starved and non-starved birds separately (Figure 6.4), the change over time was only statistically significant for non-starved birds ($R^2 = 0.64$, $F_{(2,57)} = 49.77$, $p < 0.001$). This apparent contradiction in the results may be an artefact of the two statistical methods used, in that the regression analysis gave more

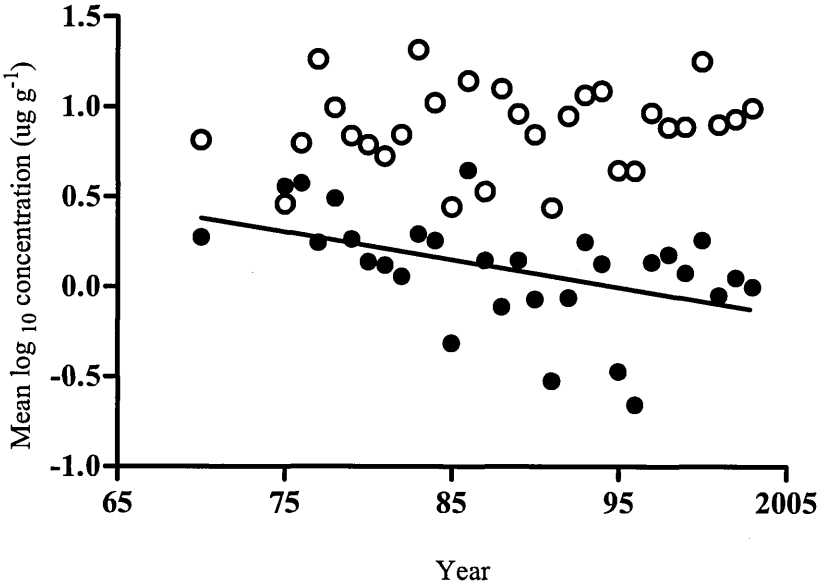

Figure 6.4 Annual geometric mean liver total PCB concentrations in starved (open circles) and non-starved (filled circles) sparrowhawks.

influence to years with greater bird numbers and may therefore be a more sensitive analysis than GLM and indicates the weak affect of time on the overall data.

In kestrels as with sparrowhawks, when data for starved and non-starved birds were analysed together, there was no evidence of any decline in liver PCB concentrations over time (Figure 6.5) ($R^2 = 0.05$, $F = 0.17$, $p > 0.01$). Analysis of the data using GLM indicates that body condition was the only significant factor affecting liver PCB concentrations in kestrels ($F_{(1,64)} = 29.24$, $p < 0.001$). Concentrations were generally higher in starved than non-starved birds although the difference between the two groups appeared less marked than for sparrowhawks (Figure 6.6). There was no evidence of any change in PCB concentrations with time for either starved or non-starved kestrels (Figure 6.6).

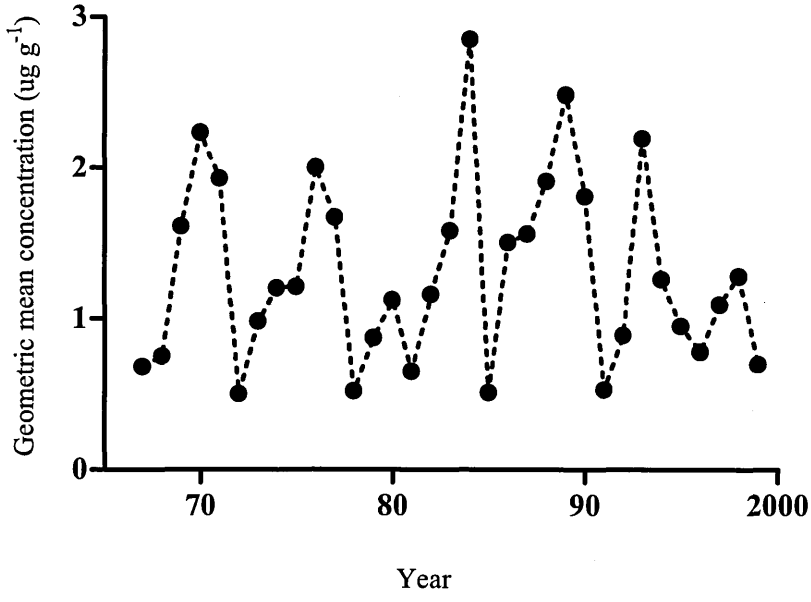

Figure 6.5 Annual geometric mean liver total PCB concentrations in kestrels between 1967 and 2003.

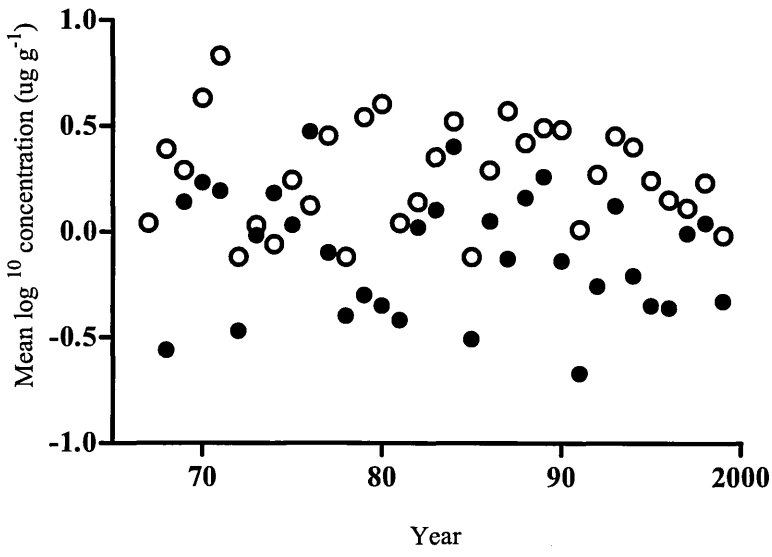

Figure 6.6 Annual geometric mean liver total PCB concentrations in starved (open circle) and non-starved (filled circle) kestrels.

6.3.2 Changes over time in the proportion of non-starved birds with low fat scores

There was an increase between 1992 and 2003 in the proportion of sparrowhawks with low (0 and 1) fat scores ($R^2 = 0.57$, $F_{(1,10)} = 13.2$, $p < 0.005$) (Figure 6.7). This would have introduced a bias towards an increase, not a decrease, in liver PCB concentrations over time. Removal of those birds with fat scores of 0 and 1 lowered the annual mean

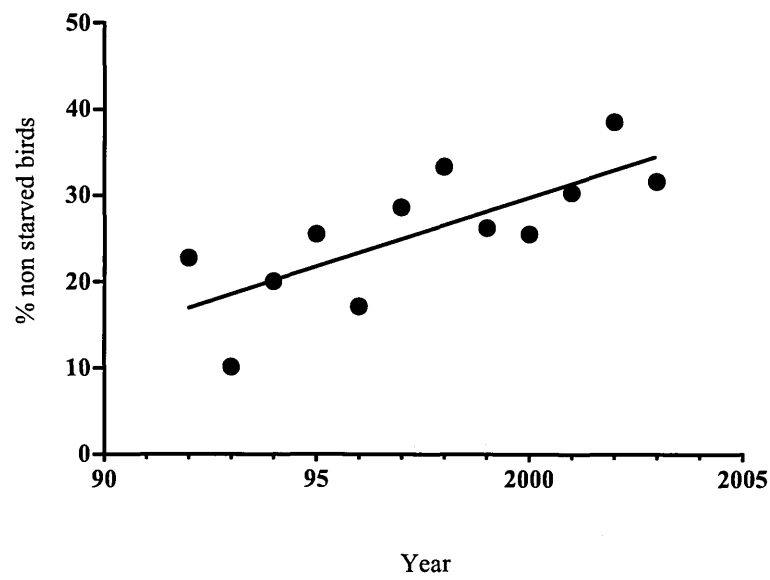

Figure 6.7 The proportion of non starved sparrowhawks with a fat score of either 0 or 1.

PCB concentrations. In contrast to sparrowhawks, the proportion of non-starved kestrels (Figure 6.8) and herons (Figure 6.9) with low fat scores did not change significantly between 1992 and 2003, and the mean (± SE) percentages of birds with low fat scores was 30% ± 4% SE and 25% ± 3%, respectively.

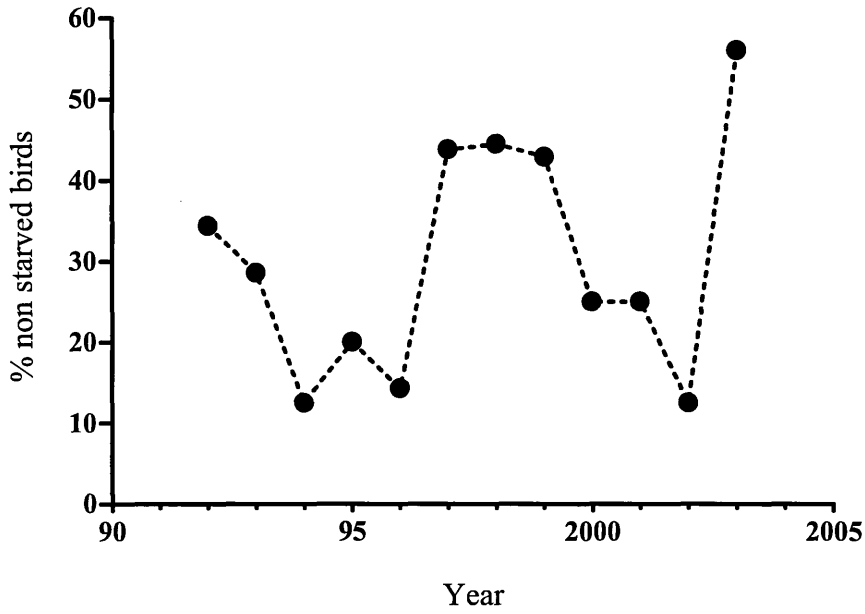

Figure 6.8 The proportion of non-starved kestrels with a fat score of either 0 or 1.

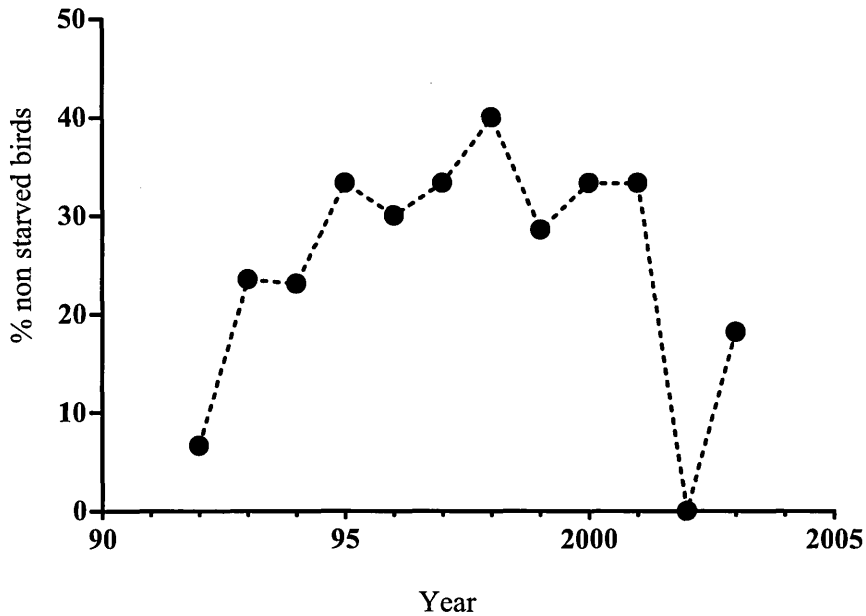

Figure 6.9 The proportion of non-starved herons with a fat score of either 0 or 1.

6.3.3 Cause of the elevation in liver PCB concentrations in starved birds

Liver weights varied significantly with fat score and were lowest in the starved birds (Figure 6.10). Non-starved (fat scores 2–5) sparrowhawks had mean liver weights that were 1.4 times greater than starved (fat scores 0 and 1) birds, ($T = 8.16$, $df=469$, $p < 0.0001$). Similarly, liver weights were 1.8 times greater in non-starved than starved kestrels ($T = 14.65$, $df = 338$, $p < 0.0001$) and 1.7 times greater in non-starved than in starved herons ($T = 5.86$, $df = 92$, $p < 0.0001$).

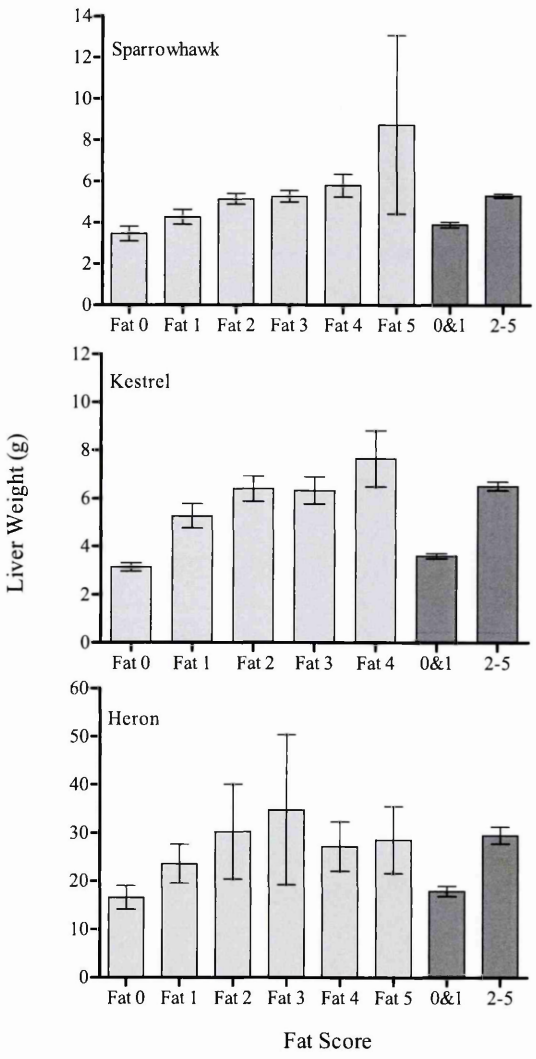

Figure 6.10 Mean ± SE liver weights of sparrowhawks, kestrels and herons with different fat scores.

The difference in mean PCB concentrations between starved and non-starved sparrowhawks was significantly greater than the change in liver weight between the two

groups (T = 7.00, $p < 0.01$, Figure 6.11). In herons and kestrels the difference in liver PCB concentrations was generally higher than the difference in liver weights between starved and non-starved birds (Figure 6.11). However, these comparisons were not statistically significant ($T < 3.05$, $p > 0.01$ for both).

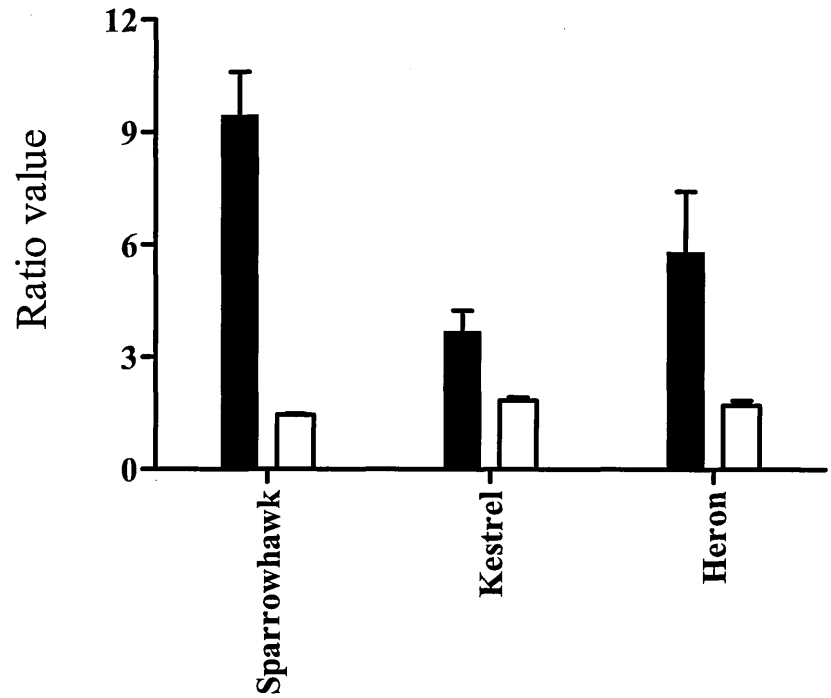

Figure 6.11 Comparison of the differences in liver PCB concentrations (black bars) and liver weights (white bars) between starved and non-starved birds.

6.4 Discussion

The results of this study clearly demonstrate that variation in nutritional state can mask detection of declining levels of environmental exposure to PCBs. When nutritional state was accounted for there was a significant decrease in liver PCB concentrations in non-starved sparrowhawks. Furthermore, the rate of change in liver PCB concentrations in herons was greater for non-starved than in starved individuals. Temporal changes in liver concentrations of other organochlorine compounds, such as DDE and HEOD, are affected in a similar manner with concentrations declining more rapidly in non-starved than in starved birds (Wienburg and Shore, unpublished data).

For the current long term analysis, cause of death was used to provide a broad indication of the likely nutritional state of the birds analysed. Detailed fat scores were not recorded prior to 1992. As a consequence of this generalised approach to assessing body condition, a proportion of the individuals identified as non-starved birds (i.e. birds that had died from causes other than direct starvation) would also have had low body fat levels. The proportion of non-starved sparrowhawks with low fat scores increased between 1992 and 2003 (the only years fat score data were available). The higher liver PCB concentrations in these birds might therefore have been expected to progressively raise the annual mean PCB concentrations of this group. Thus, the rate of decline in liver PCB concentrations in non-starved sparrowhawks that was detected (despite this bias) during this period may actually have been underestimated in recent years. The possibility that birds in poor condition have unduly influenced mean liver concentrations in non-starved birds during earlier years cannot be ruled out. Nevertheless, it is clear that variation in body fat levels between individuals did mask temporal declines in the exposure to PCBs and possibly similar lipophilic compounds.

Consistent with the earlier results (Chapter 3), body condition was the most important factor governing liver PCB concentrations, as expected; concentrations were highest in starved birds in each species. The increase in tissue concentrations of lipophilic compounds such as PCBs during periods of starvation has been well documented (Kenntner et al., 2003b; Olafsdottir et al., 1998; Elliot, 1996b; Lambeck at al., 1991; Subramanian et al., 1986) and has generally been attributed to the remobilisation from fat reserves of residues which have accumulated over an individuals lifetime. However, the lipid and protein metabolism which occurs during periods of prolonged starvation results in significant loss in body organ mass (Esselink et al., 1995), with the liver being particularly affected (Thouzeau et al., 1999). Significant losses in liver mass though not affecting the total amount of contaminant, would thus result in an increase in the

concentration of any contaminant by a similar proportion (Debacker et al., 2000; Hoffman et al., 1998; Esselinket al., 1995). Liver weights in starved sparrowhawks, kestrels and herons were almost half those of non-starved birds, a similar proportional loss to that observed in starved barn owls, *Tyto alba* (Thouzeau et al., 1999). The increase in liver PCB concentrations in starved sparrowhawks compared to concentration levels in their non-starved counterparts was greatly in excess of that which would be expected to occur through reduction in liver mass alone. This therefore suggests that sparrowhawks do indeed remobilise stored PCB residues from body fat and other tissues during starvation, and this process probably contributes to the bulk of the additional PCB burden detected in the liver of starved birds. In herons the difference in PCB concentrations in starved birds compared to non-starved birds was generally higher than might be expected through changes in liver weight alone. However the comparison may have been limited by the small sample size (n = 4) of the heron dataset and thus not be statistically significant. Hence, liver residues in starved birds are unlikely to reflect environmental changes in PCB levels (and subsequent exposure in birds) as rapidly as in non-starved birds in which liver PCB burden is presumably influenced to a much greater extent by dietary input.

In contrast to the results for sparrowhawks and herons, there was no evidence of a decline in PCB concentrations in kestrels over time, even once body condition was accounted for. Furthermore, there was no evidence that kestrels remobilise significant amounts of PCBs from body fat and accumulate the remobilised residues in the liver, because liver PCB concentrations in the starved birds increased by only by the magnitude predicted from changes in liver weight. The results described in Chapter 3 have shown that non-starved kestrels have lower liver PCB levels than either non-starved sparrowhawks or herons (see also Wienburg and Shore, 2004; Newton, 1992). Walker et al. (1987) demonstrated that kestrels have higher hepatic P450 activity than

sparrowhawks and are better able to metabolise PCBs. Thus it is possible that kestrels eliminate much of their ingested PCB burden and variation in liver residues between birds may largely reflect individual variation in detoxification capacity rather than exposure. This would indicate that kestrels may be unlikely to accumulate particularly high depots of PCB in fat. However, it is also possible that starving kestrels are capable of enhancing their PCB metabolism when challenged by increased circulating levels caused by remobilisation from fat. Whichever is the case, these results suggest that kestrels may not necessarily be a sensitive indicator species for monitoring environmental trends in PCBs and other similar lipophilic contaminants.

In conclusion, the results from this study have demonstrated that variation in nutritional state can obscure environmental trends in PCB accumulated by sparrowhawks and herons and may also delay the ability to detect significant changes in environmental levels. The influence of body condition is a major factor that needs to be considered when monitoring PCBs and similar persistent lipophilic organic pollutants in biological tissues due to the various changes in physiology that can occur during starvation. Either the nutritional state must be accounted for when analysing liver contaminant data or data analysis restricted to non-starved birds when the purpose of monitoring is to detect changes in wider environmental contaminant levels. However, although non-starved birds appear to provide a better reflection of environmental changes in PCB levels, residue data for birds in starved condition remains important as these birds may be most at risk from contaminants. This is because during starvation birds experience higher internal doses of lipophilic contaminants.

Chapter Seven

Discussion

7.1 Introduction

The concentrations at which individual PCBs occur in vertebrates arise through a combination of differences in the spatial occurrence of PCB residues, dietary exposure through the food-chain, assimilation and partitioning within the body and individual capacity to metabolise and eliminate PCB compounds. Interpretation and comparison of residue data is therefore complicated by the various factors which can influence these processes and can lead to wide variation in the concentrations of PCBs quantified in wildlife tissues. The overall aim of this study was to investigate the main causes of this variation in liver PCB concentrations in sparrowhawks, kestrels and herons from the UK and compare the magnitude and pattern of PCB contamination between the three species.

7.2 Intra-species variation in liver PCB concentrations

The results of this study have shown that the wide variation observed in liver PCB concentrations in predatory birds is explained by a combination of factors, namely nutritional state, age, and sex. Together, these factors accounted for up to half the variation in liver ∑PCB concentrations in sparrowhawks, herons and kestrels.

The nutritional status of individual birds was by far the most important factor in determining liver PCB concentrations and, as an entirely physiological factor, the influence of body condition was similar in all three predatory bird species examined. Both ∑PCB and individual PCB congener concentrations were significantly higher in starved than in non-starved birds although the extent to which congener concentrations

were affected varied between individual PCB congeners. The increase in liver PCB concentrations in birds of poor nutritional state has been thought to be primarily due to the remobilisation of previously accumulated residues from depleting fat stores during starvation (Kenntner et al., 2003b; Elliott et al., 1996; Newton et al., 1992; Lambeck et al., 1991; Subramanian et al., 1986; Cooke et al., 1979). Hence, the extent to which liver concentrations of individual congeners are affected by body condition is likely to be related to their potential to bioaccumulate. This in turn is influenced by differences in the degree to which individual congeners partition between fat and other body tissues, and the extent to which they are metabolised either following initial exposure or after remobilisation. Furthermore, the studies described in Chapter 6 indicated that nutritional state also affected liver mass, liver mass being lower in birds with smaller fat depots and lowest in birds with no visible fat depots (starved birds). There was no evidence that total PCB burdens in the liver were affected by starvation and, consequently, liver PCB concentrations were elevated in starved birds as a result of reduced liver mass. The importance of both remobilisation and organ wastage on determining liver PCB concentrations varies between species. Remobilisation was the main factor in sparrowhawks and herons whereas elevation of liver PCB concentrations in starved kestrels appeared to be mostly predominantly due to changes in liver mass. The significance of remobilisation in determining liver PCB concentrations is probably related to species differences in assimilation of and capacity to metabolise PCBs.

Age was the second most important factor explaining the variation in liver PCB concentrations. Consistent with other studies (Kenntner et al., 2003b; Johnstone et al., 1996; Platteeuw et al., 1995; Olafsdottir et al., 1995), both ∑PCB and individual congeners concentrations were typically higher in adult than first year predatory birds and probably reflected differences in the time over which adults and juvenile birds are exposed to and subsequently accumulate PCBs. Additionally, the successive increase of

PCB concentrations in birds during their first year provided further evidence for the accumulation of PCB residues over time.

Liver PCB concentrations were higher in male than female sparrowhawks and kestrels irrespective of age. Maternal transfer of PCB residues to eggs may account for differences between adult male and female birds but do not explain sex-related differences between juvenile birds that have not bred. Other sex-related differences, perhaps higher dietary intake of PCBs and/or poorer capacity to metabolise and eliminate PCBs, might account for higher liver residues in first year males than females. However, this is not consistent with the fact that the proportional increase in liver PCB concentrations associated with starvation was higher in females than males. This suggests that females may differ from males in their partitioning of PCBs between fat and other tissues, accumulating a relatively greater proportion of their ingested PCBs in fat. This could account for generally lower liver PCB concentrations in females than males and greater elevation in liver concentrations in females than males during starvation. The mechanism by which sex-dependent differences in the partitioning of PCBs between fat and other tissues is uncertain.

7.3 PCB congener profiles in British predatory birds

Whilst body condition, age and sex all influenced the magnitude of liver PCB concentrations in predatory birds, the congener profiles determined for sparrowhawks, kestrels and herons were generally similar. This presumably was largely influenced by the potential of individual congeners to undergo metabolism via the cytochrome P450 enzyme system. The congeners which were detected most frequently and at higher concentrations were those that lacked the unsubstituted *ortho-meta* and *meta-para* phenyl positions required for hydroxylation and were therefore least readily metabolised. Hence, for all three species examined, PCB congeners 138, 153, 170 and

180 contributed to up to 76% of the ∑PCB concentrations. The availability of unsubstituted phenyl ring positions for metabolism increases as the degree of chlorination decreases, consequently the tetra- and penta-chlorinated congeners were detected at lower concentrations whilst di- and tri-chlorinated congeners were rarely detected. The non-*ortho* PCBs 77, 126 and 169 were generally only detected in starved birds. Their absence in non-starved birds suggesting either exposure to these PCBs occurs at particularly low concentrations which readily partition into fat and/or they are rapidly metabolised following exposure.

7.4 Inter-species variation in PCB residues

Even when body condition, age and sex were accounted for, liver PCB concentrations still varied significantly between species. Notably, kestrels had significantly lower concentrations of both ∑PCB congeners and all individual congeners than either sparrowhawks or herons. This probably reflected their relatively high levels of hepatic monooxygenase enzymes that are involved in the metabolism of organic contaminants such as PCBs (Walker and Newton, 1997). However, kestrels feed predominantly on small mammals (Village, 1990). This is likely to result in a relatively low dietary intake of PCBs as mammals generally have a higher metabolic capacity for PCBs and other similar compounds than other vertebrates (Walker, 1998, 1990). Hence kestrels are likely to be exposed to lower concentrations of PCBs than either fish-eating species such as the heron, or sparrowhawks which feed primarily on small birds. The difference in PCB concentrations between sparrowhawks and herons varied with age and probably reflected differences in prey choice and metabolic variation within the age classes of both species.

In general, the range of PCB congeners detected and the proportional contribution of each congener to ∑PCB concentrations was similar across all three species. However,

PCB 31 was detected more frequently and PCBs 118 and 128 accounted for greater proportions of ∑PCB concentrations in herons than in sparrowhawks or kestrels. These congeners may therefore be indicative of aquatic exposure to PCBs (Hoshi et al., 1998).

7.5 Toxicity assessment of PCB exposure in British predatory birds

∑TEQ concentrations in sparrowhawks, kestrels and herons were relatively low compared to those reported in predatory birds elsewhere (Merino et al., 2005; Senthilkumar et al., 2002a, 2002b; Wiesmuller et al., 2002; Jenssen et al., 2001; Kannan et al., 2001), although they may have been underestimated to some extent in the present study as only a limited range of TCDD-equivalent compounds were quantified. Nevertheless the pattern of congener contribution to overall TEQ concentrations differed in the British birds compared to species from Europe and North America, in that congeners 118 and 169, both of which have relatively low TEF values, accounted for the bulk of liver TEQ burdens in all three of the species examined. In contrast, previous studies have reported the more toxic congeners 77 and 126 as major contributors to overall TEQ concentrations in predatory birds elsewhere (Merino et al., 2005; Wiesmuller at al., 2002; Kannan et al., 2002; Senthilkumar et al., 2002a). Hence, the exposure of predatory birds in Britain to dioxin-like PCB congeners may well be lower than elsewhere. The results of the present study also indicated that ∑TEQ concentrations in sparrowhawks, kestrels and herons were not at toxicologically significant levels as ∑TEQ concentrations were well below reported threshold values for toxicity (Bosveld et al., 2002; Hoffman et al., 1998; Elliott et al., 1996b). This assumes that PCBs are the major contributors to TEQs in sparrowhawks, kestrels and herons from Britain, however.

As with liver PCB concentrations, ∑TEQ concentrations were influenced by body condition and age. Starved birds had higher TEQ concentrations than non-starved birds whilst TEQ concentrations were higher in adults than in first year birds.

7.6 Re-evaluating temporal trends in liver PCB concentrations

This study confirmed that variation in nutritional state can obscure the detection of long-term trends in liver PCB concentrations. Once body condition was accounted for, there was a significant decrease in liver PCB concentrations in non-starved sparrowhawks and in herons, PCB concentrations declined more rapidly in non-starved than in starved birds. There was no evidence of decreasing PCB concentrations in either non-starved or starved kestrels. The elevation of liver concentrations in starved sparrowhawks and herons were above those which might be expected to occur through starvation-mediated changes in liver mass alone and were therefore likely to be the result of the remobilisation of accumulated residues from fat and other tissues. Hence, liver concentrations in starved sparrowhawks and herons did not reflect temporal changes in PCB concentrations as rapidly as non-starved birds in which liver PCB concentrations were more representative of recent dietary exposure.

There was no evidence that kestrels remobilised substantial amounts of PCBs from fat as liver concentrations in starved kestrels increased only by the magnitude that would be expected through liver wastage. This is most likely because kestrels have relatively low PCB intakes, because of their small mammal diet, and metabolise much of their ingested PCB load. Thus, variation in metabolic capacity is likely to account for a relatively high proportion of variation in liver PCB concentrations in kestrels. Given this, compared with sparrowhawks and herons, kestrels may be poor monitors for indicating temporal and spatial changes in environmental exposure to PCBs and similar compounds.

7.7 Implications for assessing and monitoring contaminant exposure in wildlife

This work has highlighted some key factors that increase the risk of toxic effects from lipophilic contaminants. Individuals and species likely to be most at risk are those that accumulate high concentrations in body tissues (particularly fat), and subsequently face periods of limited prey availability and/or high energy requirements that result in significant loss in body fat reserves. Birds are likely to experience various periods of poor nutritional state during their lifetime. This may be a consequence of low food availability and may also occur during specific, energetically demanding, physiological processes, such as breeding, moult and migration (Village, 1990; Newton, 1986; Dyrcz, 1987). The latter are periods of high physiological stress. The additional impact of acute exposure to contaminants remobilised from fat during these periods has not been previously determined, although contaminants were implicated in a seabird mortality incident that occurred during moult (Henny et al., 1995). Diseased individuals may likewise be particularly susceptible to toxicological stress and experience increased hepatic concentrations of PCBs and similar compounds if the pathology causes loss of body condition. Overall, the influence of nutritional state on liver PCB concentrations is likely to be most pronounced in species and individuals with relatively low capacity to metabolise contaminants. This is because organ concentrations are increased not only as a result of organ wastage (which will occur in all individuals irrespective of their detoxification capacity) but also due to the release of previously assimilated residues that have been stored in fat. The importance of body condition in mediating organ contaminant concentrations means that species that undergo large-scale variation in fat deposition and utilisation, for example hibernating species, may be at particular risk when fat reserves are almost completely exhausted.

Changes in body condition are likely to impact in a similar manner on the tissue concentrations of other potentially harmful lipophilic contaminants which readily accumulate in tissues. Such compounds include dioxins, polybrominated biphenyls and polychlorinated naphthalenes amongst others, all of which can be accumulated by predatory birds (Elliott et al., 2005; Jimenez et al., 2005; Kannan et al., 2001). Hence individuals in poor nutritional state will be exposed to the highest liver concentrations of a range of toxic contaminants, not just PCBs. This may give rise to additive toxicity, for example where effects are mediated through the *Ah*-receptor, and/or independent toxicity where multiple toxic pathways are affected.

The impacts that age and sex had on liver PCB concentrations varied between sparrowhawks, herons and kestrels. Thus, they appear to be species-specific rather than general in their impacts. This is probably because effects are most likely mediated through age/sex-related variation in dietary PCB intake and metabolic capacity and the relative importance of these factors is likely to differ between species. Nevertheless these factors have the potential to influence the exposure and assimilation of birds and other vertebrates to a wide range of both pollutants. Therefore, accurate risk assessment requires that differences in body condition, age and sex of individuals are considered when identifying those groups most at risk from the toxic effects of chemical exposure.

This study further demonstrated that intrinsic physiological factors can have a confounding influence on the ability to detect trends and patterns in contaminant levels when using biological tissues for monitoring. As a result, the extent or rate of changes over time and space in the assimilation of environmental contaminants in body organs may be obscured or underestimated. This is likely to be more pronounced for lipophilic than inorganic contaminants because the effects on organ concentrations of remobilisation of lipophilic contaminants are additional to those caused solely by organ wastage. Such impacts may have particular significance when monitoring newly-

identified chemicals of concern, because they may delay early detection of increasing exposure. This is because *accurate* assessment of baseline concentrations is likely to prove problematic and intra-year variation may be high relative to inter-year changes. Large-scale and long-term monitoring schemes, such as the PBMS, the monitoring of contaminants in UK otters (Simpson et al., 2000), and schemes that use residue magnitude to determine xenobiotics that have been the cause of death (Barnett et al., 2004), do not typically take body condition into account but clearly need to do so.

Finally, the differences detected between kestrels and either sparrowhawks or herons in their pharmacokinetic handling of PCBs emphasises the importance of selecting suitable species for monitoring. Measuring PCB concentrations in kestrels demonstrates that British populations of this species are not exposed to harmful PCB concentrations, but these findings are not necessarily representative of potential impacts in other species.

7.8 Further research needs

This study has not attempted to investigate the importance of spatial variation in PCB exposure to determining tissue concentrations in sparrowhawks, kestrels and herons. The birds analysed for PCB contamination in this study were from widespread locations throughout Britain. Initial studies (outside the scope of this thesis) using Geographical Information Systems (GIS) have indicated that there may be contamination 'hotspots' for PCBs in birds (Broughton et al., 2003). These are probably quite rare but PCB exposure in predatory birds in Britain may also vary to some extent with latitude, rainfall, and land use (Shore et al., 2006). Although the results from this study suggest that a substantial proportion of the intraspecific variation in liver PCB residues is due to variation in body condition, age and sex, at least 50% of the variation remains unexplained. The degree to which differences in spatial location, diet and metabolic capability explains the reminder of the variation is unclear.

Toxicity threshold levels for TEQ exposure in birds are limited and generally either derived from studies on healthy chicks (Bosveld et al., 2000; Elliott et al., 1996). The overriding evidence from the present work is that birds in poor nutritional state are likely to be exposed to some of the highest liver concentrations of PCBs and other lipophilic compounds. The extent to which individuals under such physiological stresses are capable of mounting a metabolic response to contaminants is uncertain. Induction of P450 enzymes is usually reflected by an increase in liver mass yet, as shown by the present studies, starvation is associated with a decrease in liver mass. Thus, it is not clear if enzyme induction occurs fully in starving birds that are undergoing organ wastage. If not, starvation may be associated both with high concentrations of lipophilic contaminants in target organs (due to remobilisation) and simultaneous impairment of detoxification processes. Further studies are needed to evaluate the impact of starvation and other physiological stressors on the toxicity of PCBs and other lipophilic contaminants. In particular, such work may need to focus on whether no observable effect concentrations (NOEC) for PCBs and other persistent pollutants need to be modified for animals in poor physiological condition.

7.9 Conclusions

This study has quantified for the first time the importance of intrinsic physiological factors in determining liver concentrations of PCBs in predatory birds. Individuals in poor nutritional state exposed to elevated PCB concentrations and TEQ concentrations are likely to be at most risk from PCB toxicity. The results presented in this thesis indicate that sparrowhawks, kestrels and herons from Britain are currently unlikely to be exposed to toxicologically significant concentrations of PCBs, but have highlighted the importance of accounting for intra-specific variation in tissue contaminant concentrations when monitoring trends in contaminant exposure in wildlife and when assessing the associated likely toxic impacts.

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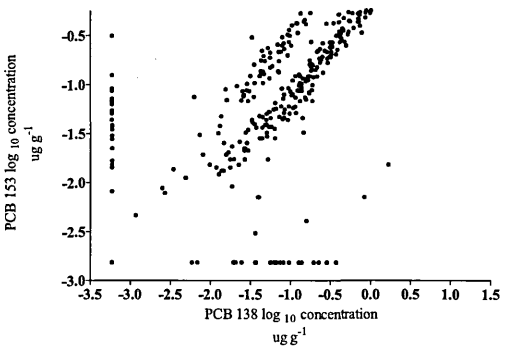
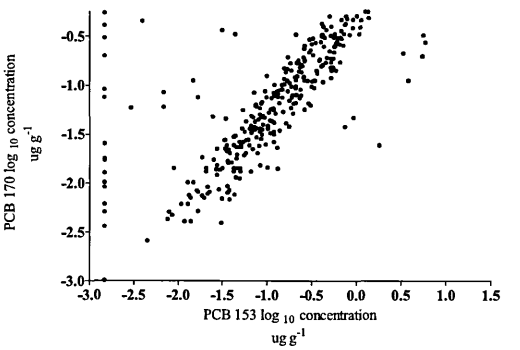
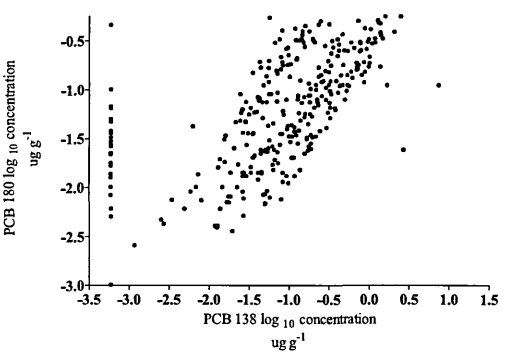
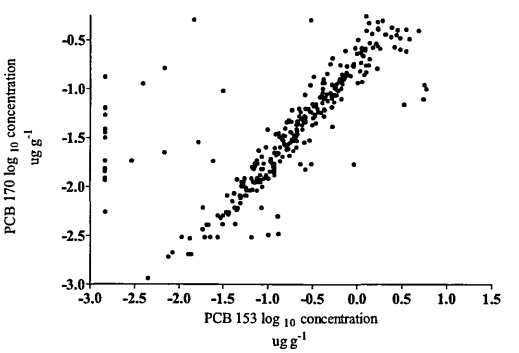
Appendix 1

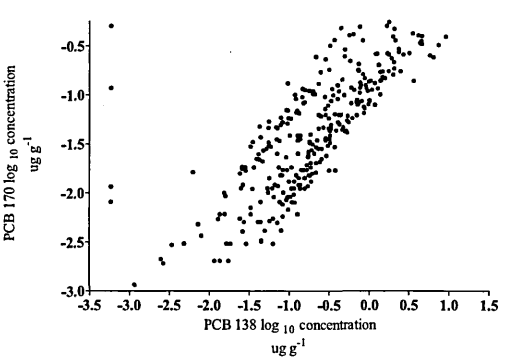
A1.1 Correlations between liver PCB congener concentrations in sparrowhawk livers

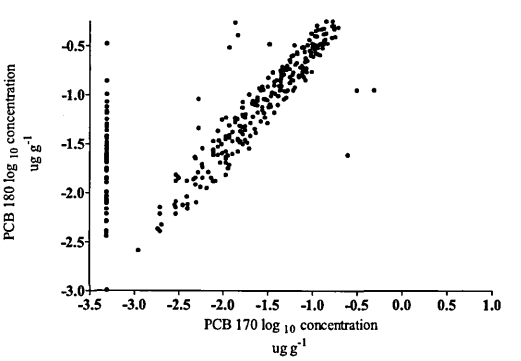


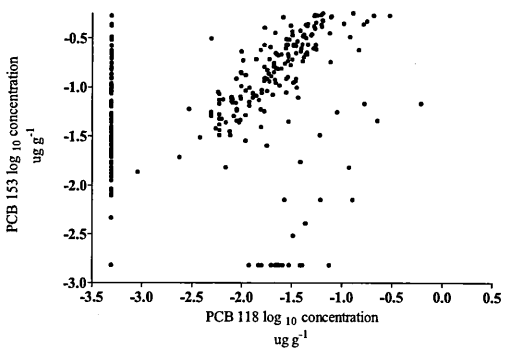








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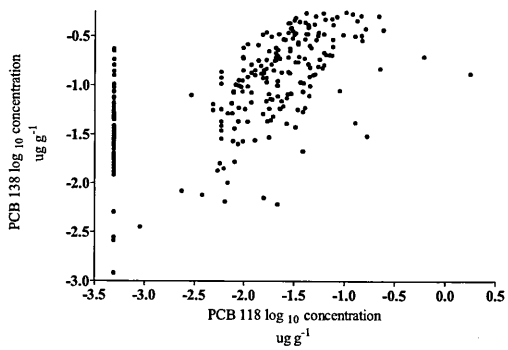
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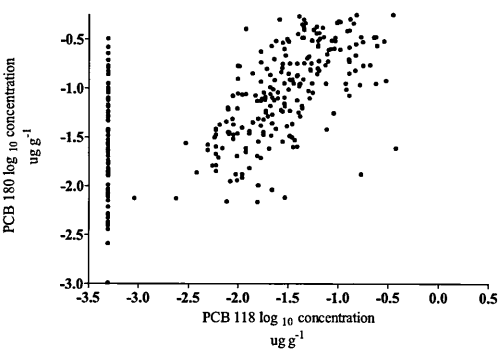
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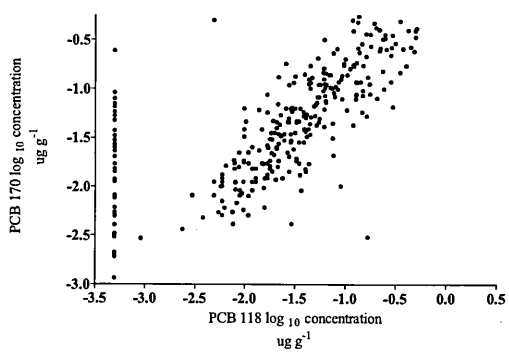
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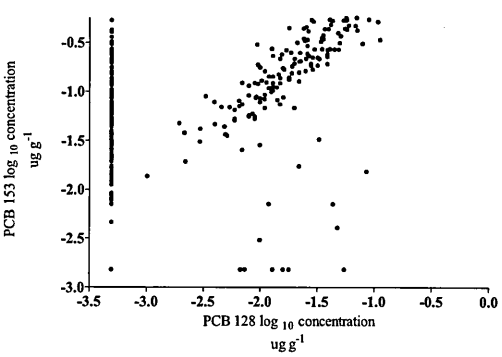
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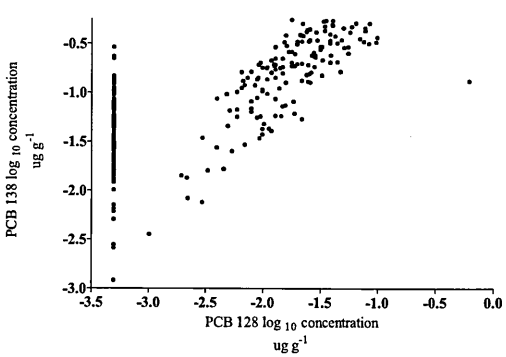
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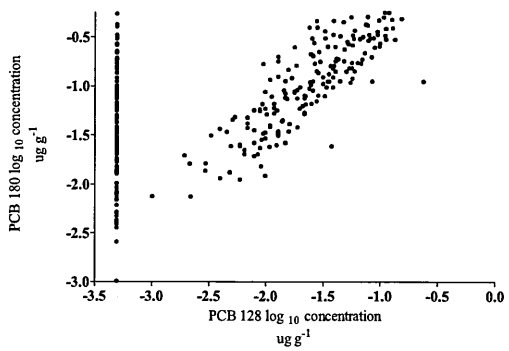
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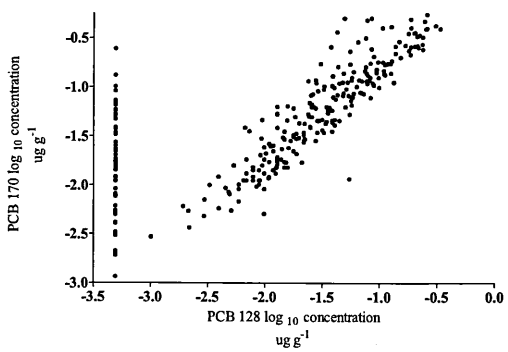
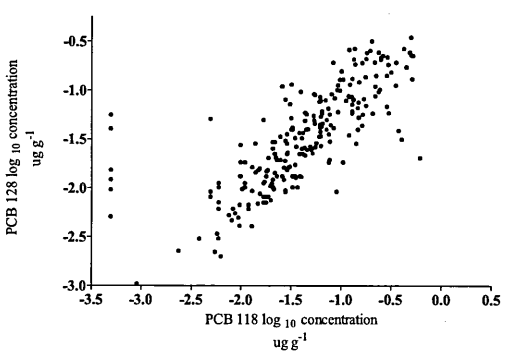
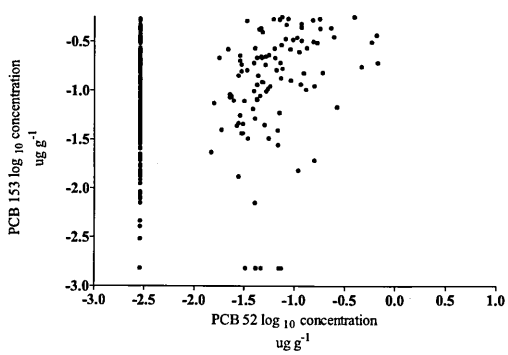
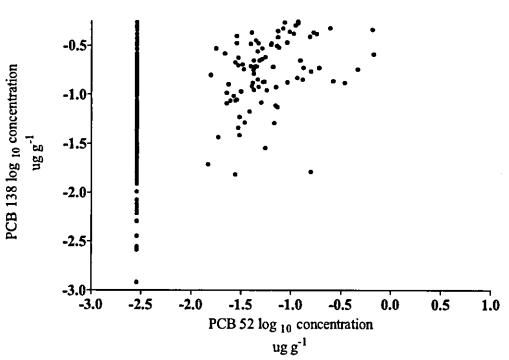
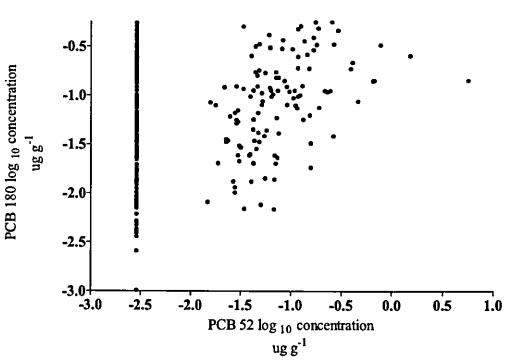
PCB 128 log 10 concentration
ug g-1

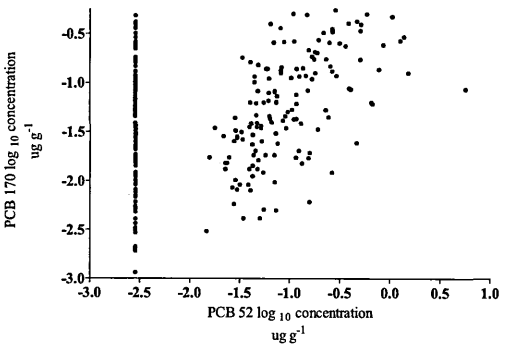

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0
PCB 128 log 10 concentration
ug g-1

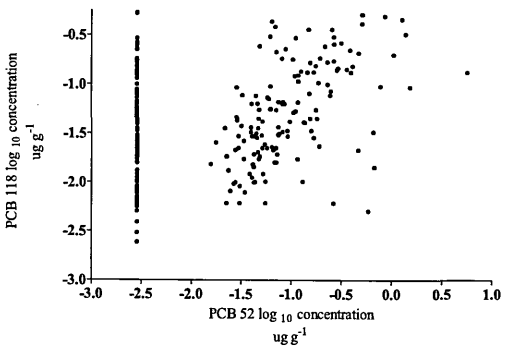

PCB 128 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5
PCB 118 log 10 concentration
ug g-1

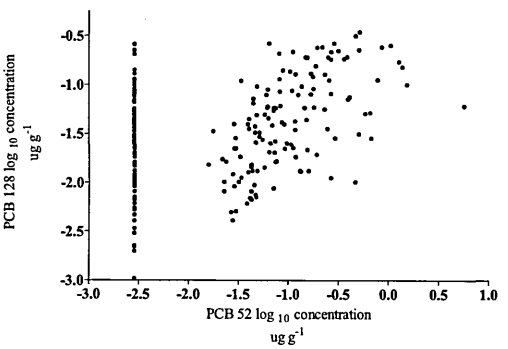

PCB 153 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

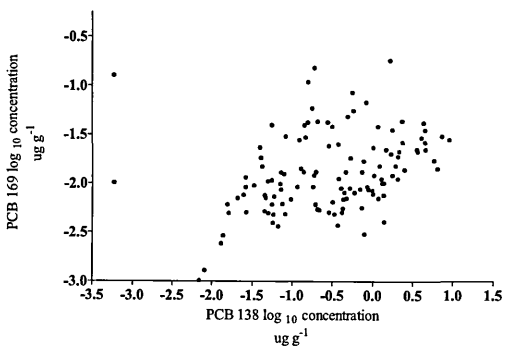

PCB 138 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

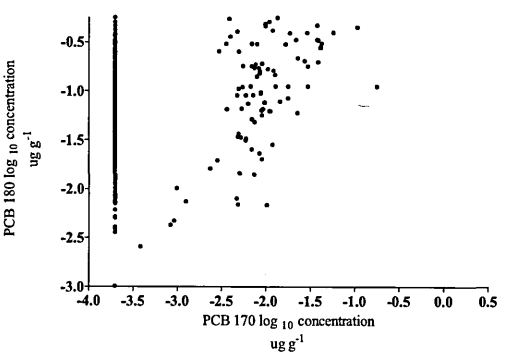

PCB 180 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

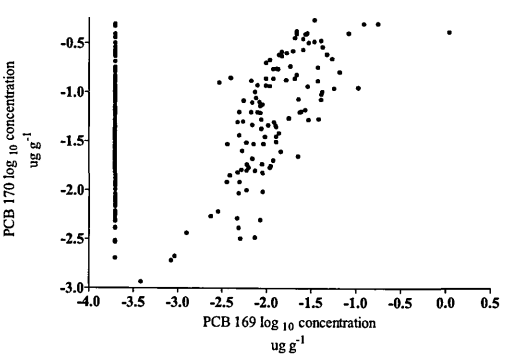

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

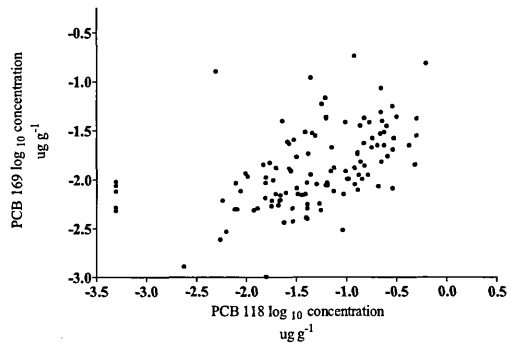

PCB 118 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

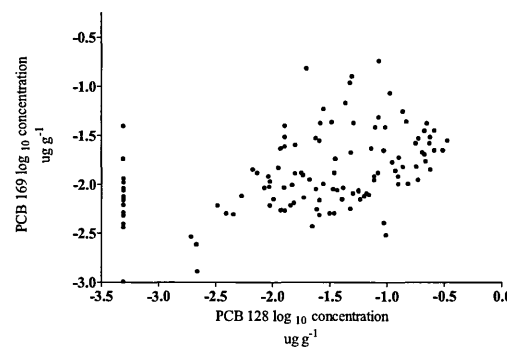

PCB 128 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

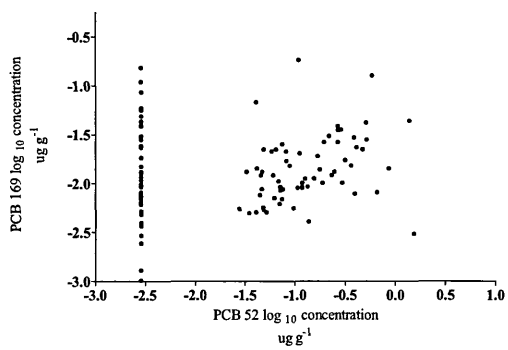

PCB 169 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
PCB 138 log 10 concentration
ug g-1

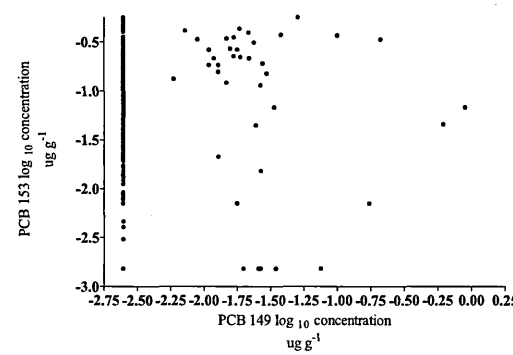

PCB 180 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-4.0 -3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5
PCB 170 log 10 concentration
ug g-1

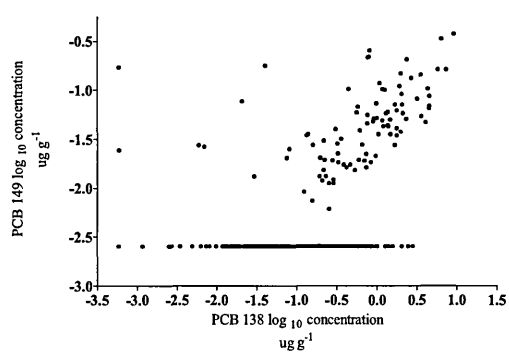

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-4.0 -3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5
PCB 169 log 10 concentration
ug g-1

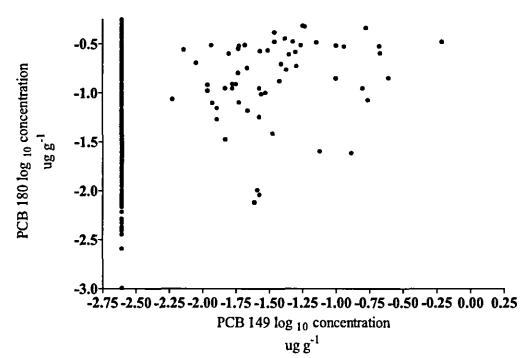

PCB 169 log 10 concentration ug g-1
PCB 118 log 10 concentration ug g-1

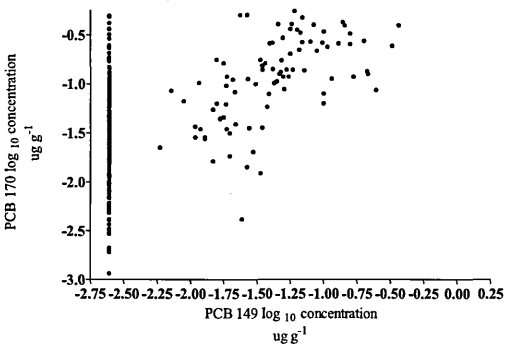

PCB 169 log 10 concentration ug g-1
PCB 128 log 10 concentration ug g-1

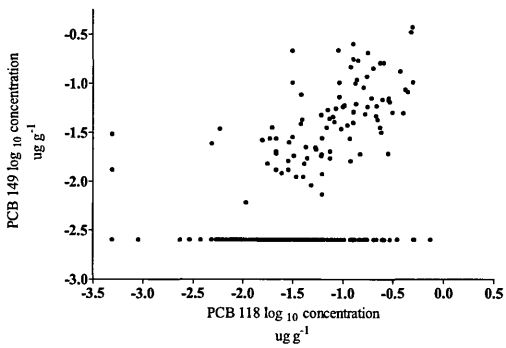

PCB 169 log 10 concentration ug g-1
PCB 52 log 10 concentration ug g-1

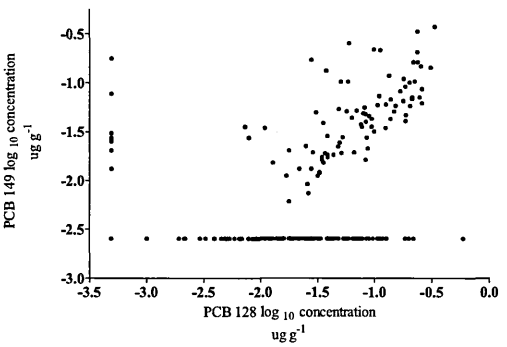

PCB 153 log 10 concentration ug g-1
PCB 149 log 10 concentration ug g-1

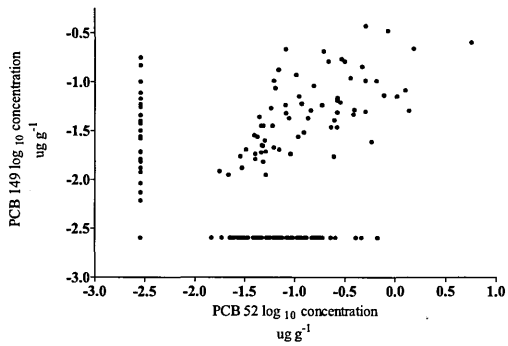

PCB 149 log 10 concentration ug g-1
PCB 138 log 10 concentration ug g-1

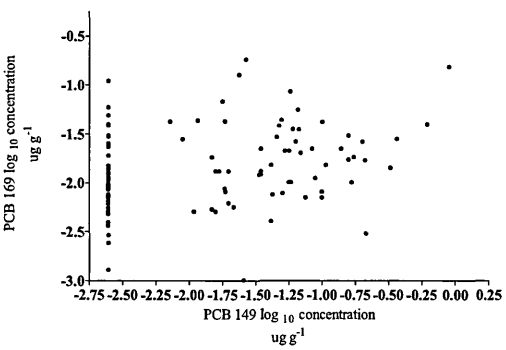

PCB 180 log 10 concentration ug g-1
PCB 149 log 10 concentration ug g-1

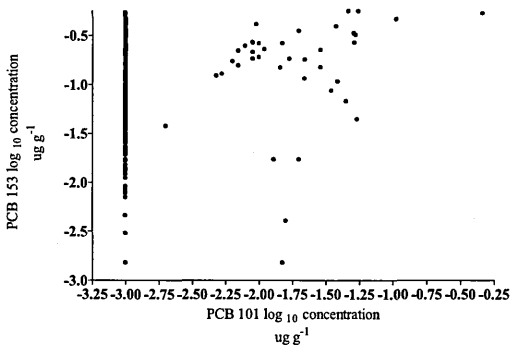

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 149 log 10 concentration
ug g-1

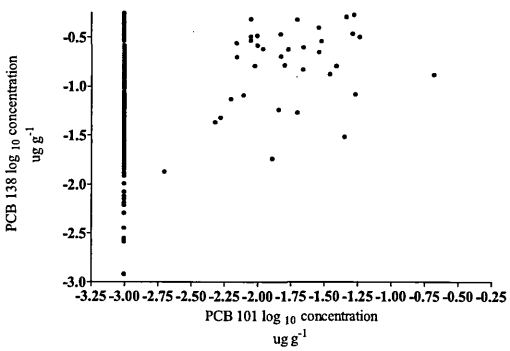

PCB 149 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5
PCB 118 log 10 concentration
ug g-1

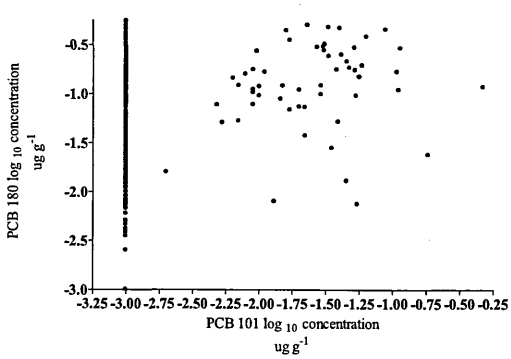

PCB 149 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0
PCB 128 log 10 concentration
ug g-1

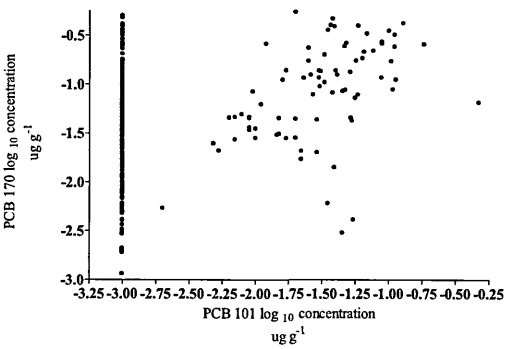

PCB 149 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration
ug g-1

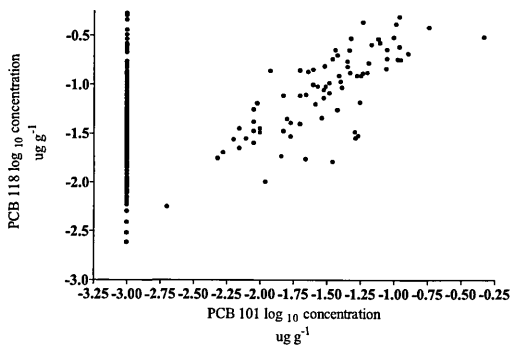

PCB 169 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 149 log 10 concentration
ug g-1

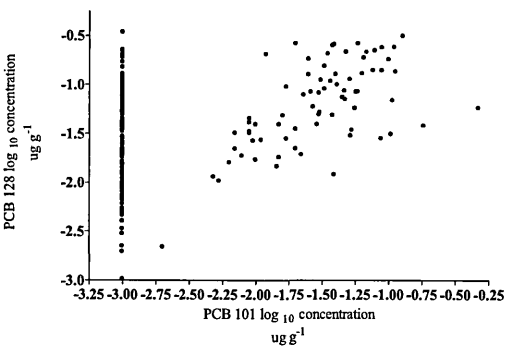

PCB 153 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration
ug g-1

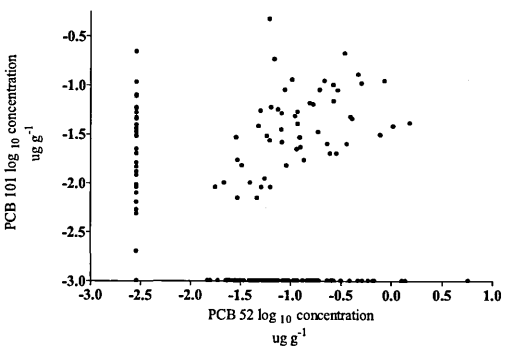

PCB 138 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration ug g-1

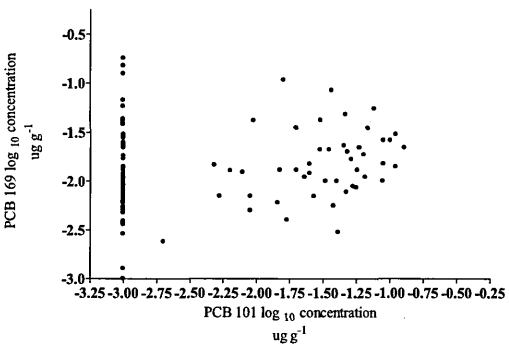

PCB 180 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration ug g-1

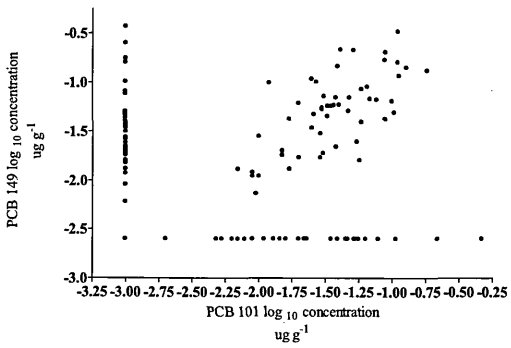

PCB 170 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration ug g-1


PCB 118 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration ug g-1

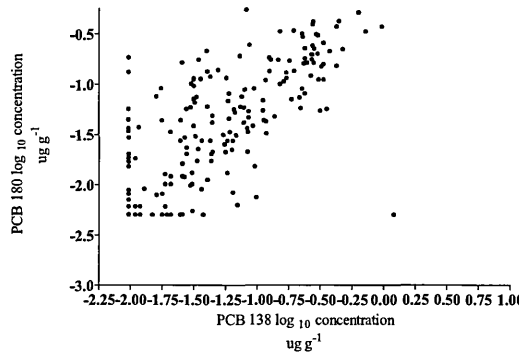

PCB 128 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration ug g-1

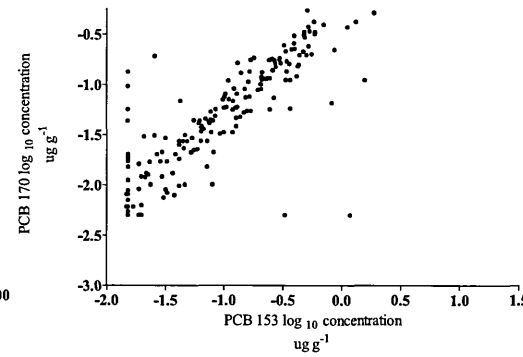

PCB 101 log 10 concentration ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0
PCB 52 log 10 concentration ug g-1

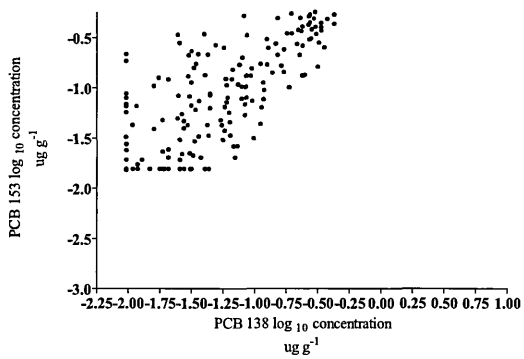

PCB 169 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration
ug g-1

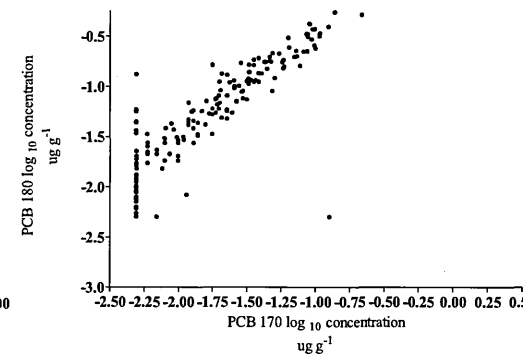

PCB 149 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.25 -3.00 -2.75 -2.50 -2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25
PCB 101 log 10 concentration
ug g-1

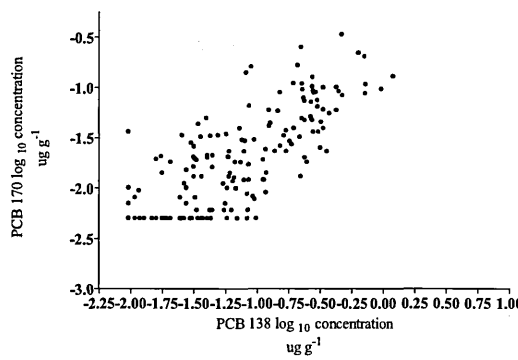
A1.2 Correlations between liver PCB congener concentrations in kestrel livers.

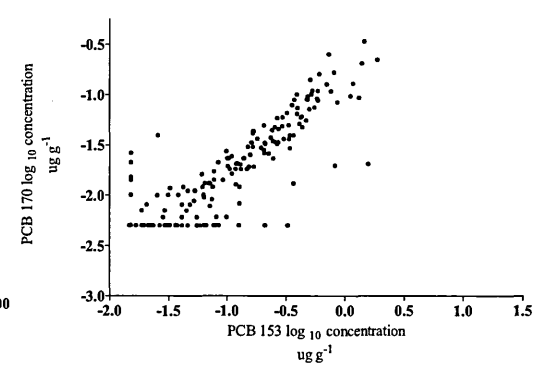


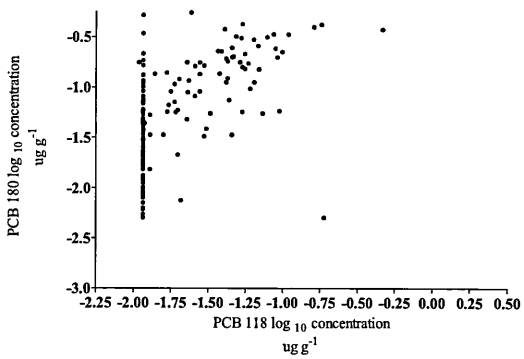


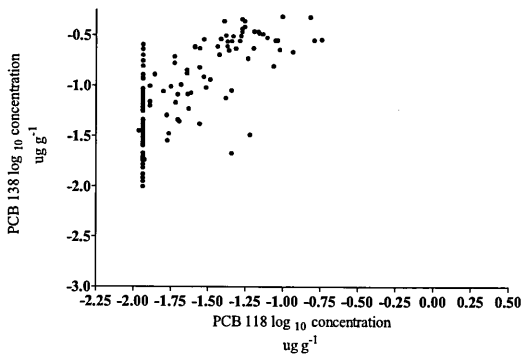


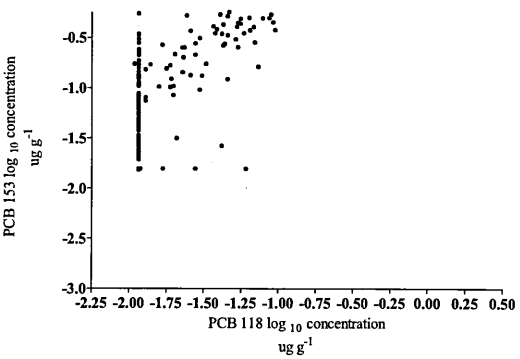


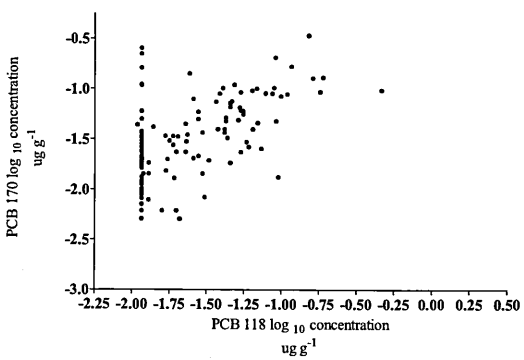


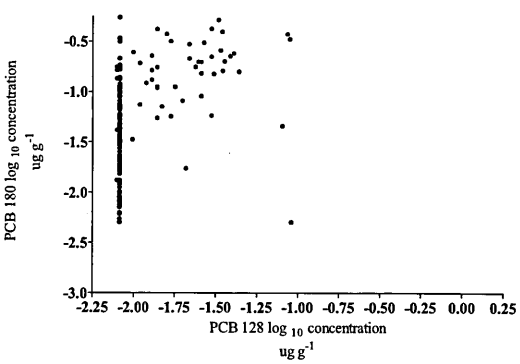


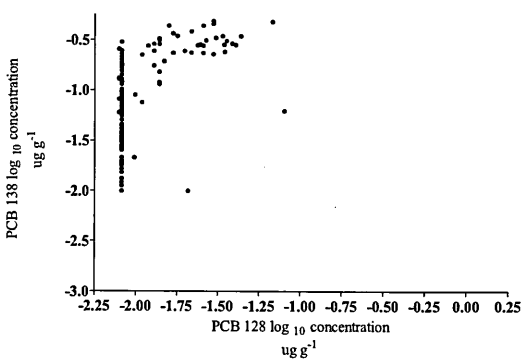

PCB 180 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25 0.50
PCB 118 log 10 concentration
ug g-1

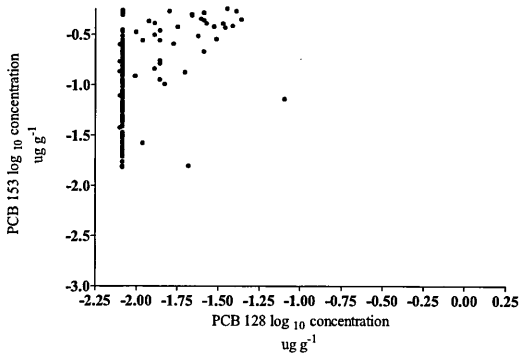

PCB 138 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25 0.50
PCB 118 log 10 concentration
ug g-1

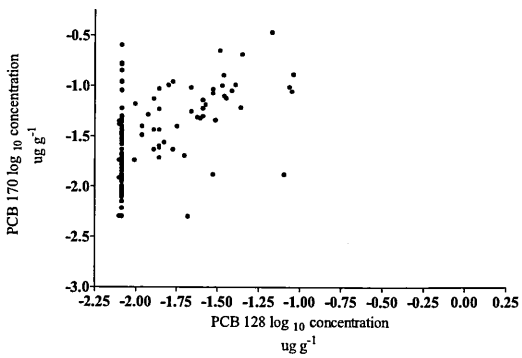

PCB 153 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25 0.50
PCB 118 log 10 concentration
ug g-1

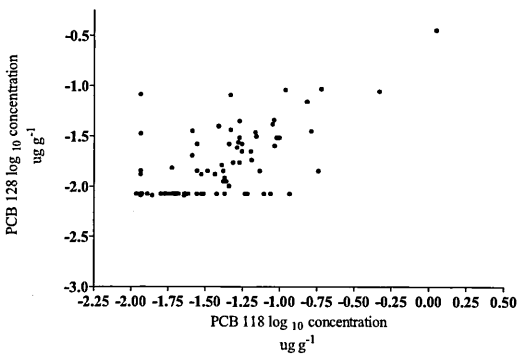

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25 0.50
PCB 118 log 10 concentration
ug g-1


PCB 180 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 128 log 10 concentration
ug g-1

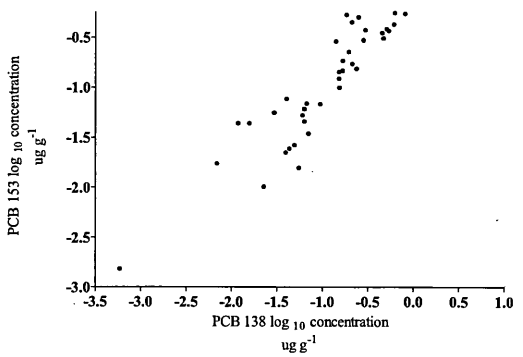

PCB 138 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 128 log 10 concentration
ug g-1

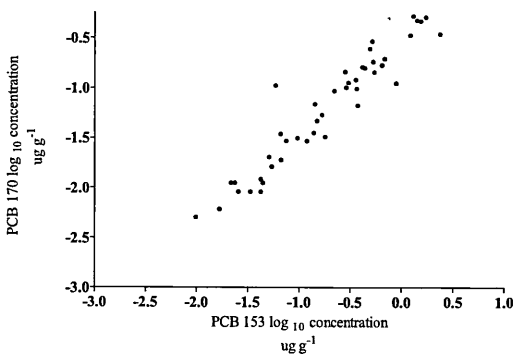

PCB 153 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 128 log 10 concentration
ug g-1

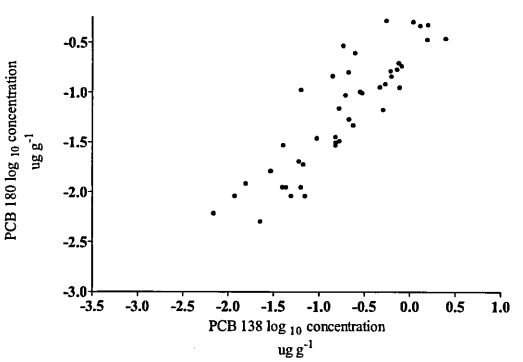

PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25
PCB 128 log 10 concentration
ug g-1

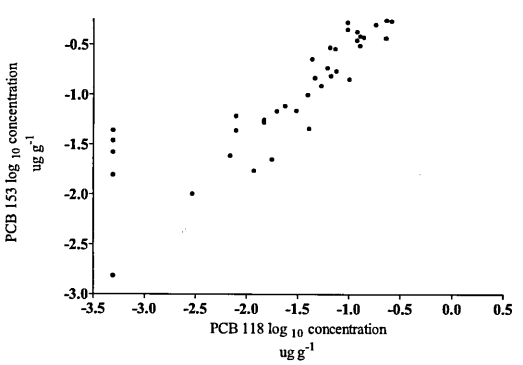

PCB 128 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-2.25 -2.00 -1.75 -1.50 -1.25 -1.00 -0.75 -0.50 -0.25 0.00 0.25 0.50
PCB 118 log 10 concentration
ug g-1

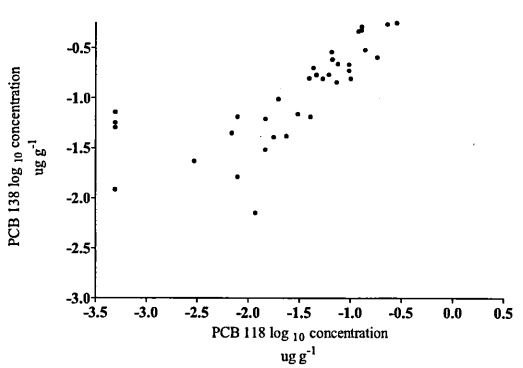
A1.3 Correlations between liver PCB congener concentrations in heron livers.

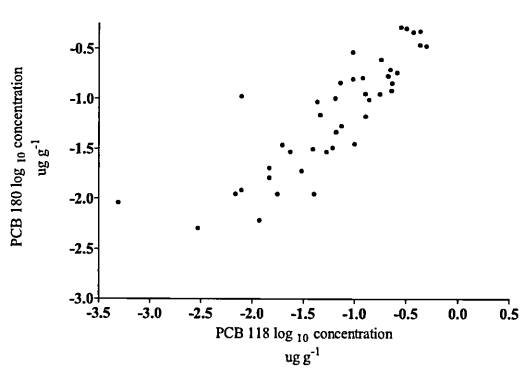


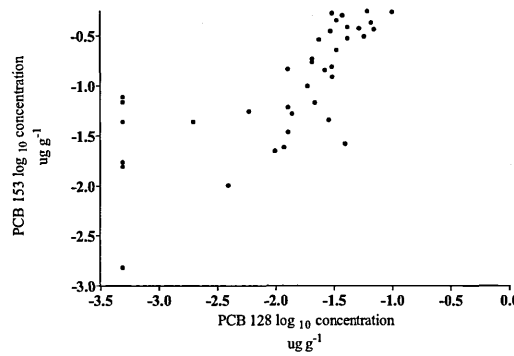


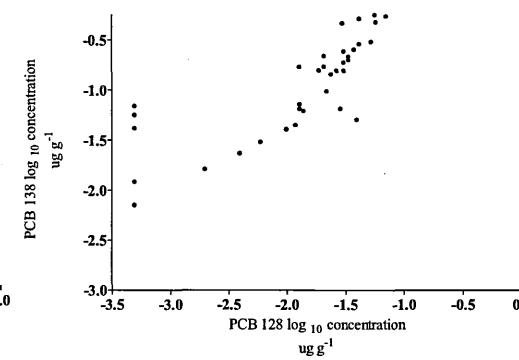


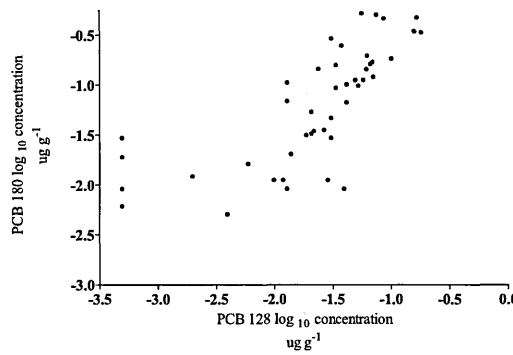


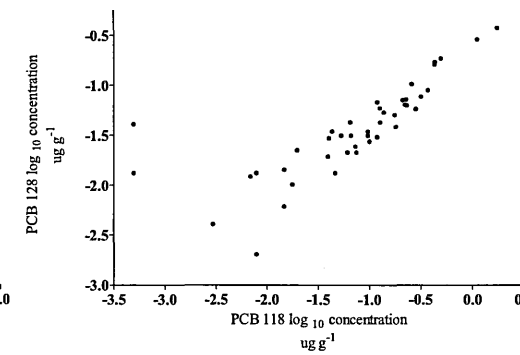


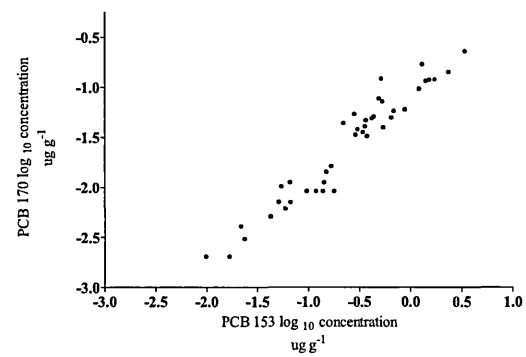


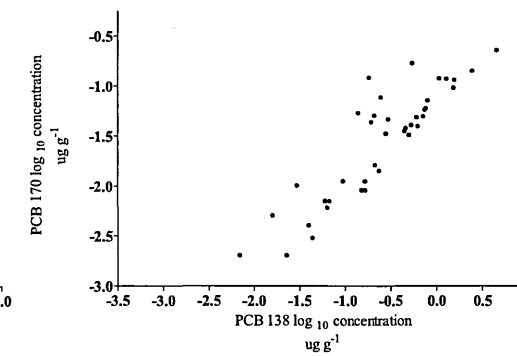

PCB 153 log 10 concentration
ug g-1
PCB 128 log 10 concentration
ug g-1

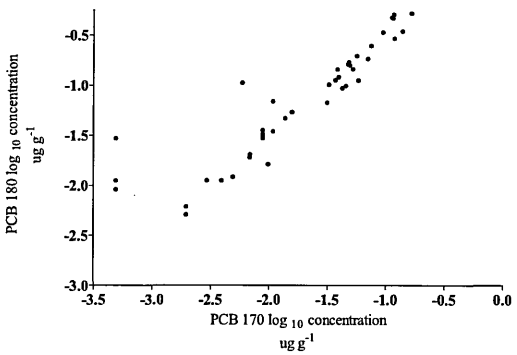

PCB 138 log 10 concentration
ug g-1
PCB 128 log 10 concentration
ug g-1

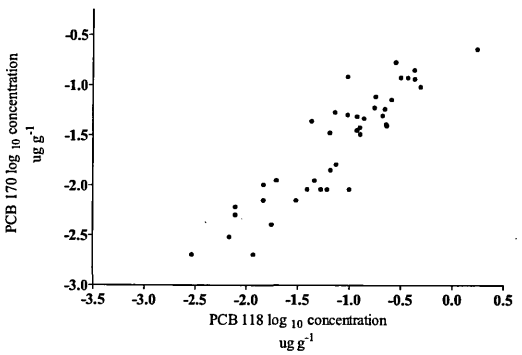

PCB 180 log 10 concentration
ug g-1
PCB 128 log 10 concentration
ug g-1

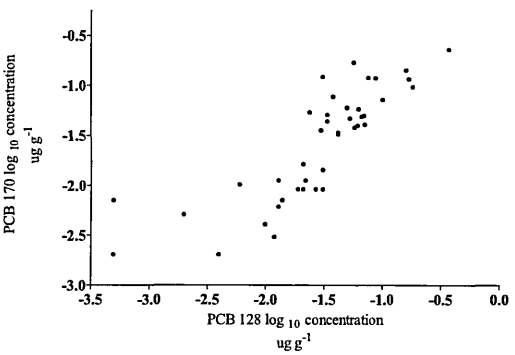

PCB 128 log 10 concentration
ug g-1
PCB 118 log 10 concentration
ug g-1


PCB 170 log 10 concentration
ug g-1
PCB 153 log 10 concentration
ug g-1


PCB 170 log 10 concentration
ug g-1
PCB 138 log 10 concentration
ug g-1


PCB 180 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0
PCB 170 log 10 concentration
ug g-1


PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5
PCB 118 log 10 concentration
ug g-1


PCB 170 log 10 concentration
ug g-1
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5 -3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0
PCB 128 log 10 concentration
ug g-1